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A few months' relief, but what then?

So, at the eleventh hour, a way has been found for the Science Research Council (SRC) to pay its international subscriptions, notably to CERN, without thereby bankrupting all its other activities. It isn't clear as yet that the hat which had embarrassingly to be passed round all manner of scientific establishments for donations to the cause will be passed back intact, but there is hope that the modest sum that was found therein will eventually get back to the kindly oceanographers, invertebrate virologists and so on who did their bit. But at the risk of boring our readers even more—and we promise to say nothing further on the subject in 1976—there are still some unanswered questions about the future of international subscriptions in times of inflation and a sliding exchange rate.

The somewhat disturbing truth about the resolution of the problem for this financial year (to March 31, 1977) is that it has been possible only by some dexterity in shuffling funds and reinterpreting rule books at the Department of Education and Science (DES) and the Treasury. There had always been a feeling around that the cash-limits problem could be circumvented somehow, and so it turned out. Some money (about £2.5 million) which had been earmarked for university purposes in the form of an operational reserve was early on seen to be spare and the Treasury had pointed this sum at the SRC. But the real breakthrough came when some very careful reading of the rulebooks, and, one suspects, some very slight reinterpretation of them, showed that a sum of £1.6 million previously thought to be the responsibility of the research councils was found to be chargeable to other departments involved in commissioning research in council establishments. Not £1.6 million was saved that way, but double it!

If this means a somewhat relaxed Christmas for all concerned, it doesn't necessarily mean good news for the

financial year 1977–78. In the next few weeks the results of Britain's widely leaked commitment to the International Monetary Fund to reduce public spending in exchange for a large loan are bound to trickle through the Whitehall machine. It would be foolish to think that in some way science can be buffered from these cuts, either in the sectoral departments with a science and technology constituent or through the Department of Education and Science. All of which means that there are not only bound to be several uneasy months as decisions are made on whether staff levels are to be maintained or whether the cuts are to be passed on to sub-contractors, university departments and the like; it also means that the monolithic international subscriptions, not open to little bits of trimming here and there, are bound to become more exposed, occupying, as they will, a growing fraction of SRC's budget. And the idea of strict cash limits, which has been at the root of this year's difficulties, is likely to be around for years to come judging by the general enthusiasm in Whitehall for them.

Thus, although this year's patching-up operation is welcome indeed, the lack of any sort of systematic resolution of the problems brought on by variable exchange rates will leave us with a perennial headache, unless the pound starts to move the other way. The waste of time, the stirring up of animosities, the internal conflict that followed this year's difficulties are not things that ought to become an annual feature of the life of practitioners of big science. It still seems to us most desirable that within the near future a mechanism will be found by which international subscriptions are not jeopardised by currency fluctuations. Other countries manage it—surely if DES contains people clever enough to convert to a £1.6 million bill into a £1.6 million IOU, it contains people clever enough to devise means of saving themselves the bother in future years. □



The breeder reactor: a fossil fuel viewpoint

David Merrick considers what economic and technical factors would make the fast breeder reactor a viable option

THE concept of the fast breeder reactor is elegant and simple: to generate electricity and, at the same time, to produce additional fuel from the uranium discarded by the existing thermal reactor system. Without the breeder reactor, it seems likely that the role of nuclear energy will begin to be constrained by the price and availability of uranium at about the turn of the century. There is, however, no consensus on the desirable, or even possible, contribution of breeder reactors to future energy supplies. Foremost among the uncertainties discussed in the current debate are the questions of safety and environmental impact. Assuming concern in these areas can be satisfactorily and economically resolved, there still remain questions on the technical and economic limitations of the use of breeder reactors.

Performance of the breeder system

In making the case for the breeder reactor, the most commonly quoted index of performance is that 30% to 60% of the energy available in the uranium fuel can be liberated, compared with about 1% in most thermal reactors. The exact value of the efficiency depends on the plutonium losses in reprocessing, the 60% figure being valid only if these can be limited to 2%.

In addition to approximately 4 tonnes of plutonium in the core, a 1 MW breeder reactor contains about 50 tonnes of depleted uranium in the core and blanket. This is converted by the reactor to plutonium (and other elements) and typically, a further 50 tonnes of depleted uranium are required as replacement during a lifetime's operation. A single breeder reactor will, therefore, utilise only 15% to 30% of the uranium required for its operation, if the initial charge is included.

The uranium inventory can be considered as a form of storage and remains useful provided there is a continuing programme of breeder reactor construction. If, however, an expanding programme of breeder capacity is planned, a further constraint, the availability of fissile material for the reactor core, is encountered. Although it would be technically possible to use highly

enriched uranium for this purpose, to do so would be costly, and the reactor performance, although improving slowly during the lifetime of the plant, would not initially be in the 'breeding' regime¹. For these reasons the design of breeder reactors, and scenarios for their introduction (for example the United Kingdom Atomic Energy Authority's 1974-75 evidence to the Royal Commission on Environmental Pollution²), concentrate on the use of plutonium as the fissile material.

It is expected that the Liquid Metal Fast Breeder Reactor (LMFBR) will breed 20%-25% more plutonium than is required as fuel. This excess plutonium can be used as inventory for further breeder reactors. A crucial parameter affecting the strategy for the introduction of breeder reactors is the doubling time, that is, the time taken for a breeder reactor to produce sufficient plutonium to provide the initial fuel charge for another similar reactor. The doubling time depends not only on the breeding gain of the reactor itself, but also on the delay time and losses in reprocessing the partially burned fuel. At the present state of reactor and reprocessing technology, the doubling time would be more than 50 years³, although this is expected to be cut to a period in the range 25 to 40 years for commercial reactor systems (at a 70% load factor). As neither the reactor nor the reprocessing plant are likely to be demonstrated on a commercial scale for several years, it seems prudent to assume a mid-range value of, say, 33 years for planning purposes.

It is claimed that further reductions in the doubling time could be obtained by using carbide instead of oxide fuel, or a gas-cooled reactor instead of the present liquid metal designs. However, even the achievement of a 33-year doubling time in an acceptable commercial system will entail a substantial development programme, and whether sufficient incentive would then remain to embark on further such programmes is not clear.

At the beginning of a programme of breeder reactor construction, the plutonium required for the reactor core would be obtained as a by-product of the thermal reactor programme. In most situations, the available stocks of plutonium would be rapidly exhausted, and the rate of construction of breeder

reactors would then be limited by the rate at which plutonium becomes available from existing thermal and breeder reactors. In the UK, for example, stocks of separated plutonium would, at present, enable 1 GW of breeder capacity to be built². This should rise to about 7 GW in 1985.

Whether the breeder programme can ever become independent of the thermal reactor programme depends on the growth rate of the nuclear electricity system, and the doubling time. At a doubling time of 33 years, plutonium production from the breeder reactor system can only sustain a growth rate of around 3% per annum. For the growth rate of the nuclear electricity system to be greater than 3% per annum, it is necessary to construct both thermal and breeder reactors.

In this situation, the efficiency with which uranium is utilised by the overall system of breeder and supporting thermal reactors is considerably lower than the value of 60% claimed for the breeder reactor alone. This is illustrated in Fig. 1, which shows the uranium utilisation for the system of breeder (LMFBR) and supporting thermal reactors (PWR) at various growth rates. Also shown, for comparison, is the uranium utilisation if the CANDU thermal reactor, with plutonium recycle, were to be used. This system performs better than the LMFBR/PWR system at growth rates above about 5% per annum.

If, therefore, a rapid build-up of nuclear capacity were desirable, the LMFBR/PWR system would, during the period of growth, offer a relatively small improvement in uranium utilisation over the PWR, and little or no improvement over the CANDU system. Only if the projected plutonium doubling time of commercial breeder reactor systems were to be substantially reduced could a high growth rate in breeder capacity be achieved, but there is no evidence at present that this would be technically or economically possible.

Growth of the breeder system

Besides limiting the overall efficiency of uranium utilisation in the nuclear reactor system, the availability of plutonium for the cores of breeder reactors

Table 1 Estimated uranium import requirements 2000-2020 AD

Growth rate (% per annum)	LWR + LMFBR	LWR + Pu recycle	CANDU + Pu recycle
1	39,000	105,000	51,000
3	76,000	214,000	103,000
5	220,000	433,000	208,000

also acts as a constraint on the rate at which breeder capacity can be introduced. This can be illustrated by considering an idealised situation in which the commissioning of new power stations precisely matches the steadily growing level of demand. The maximum rate of introduction of breeder reactors is shown in Fig. 2 if, at year zero, new power station construction is suddenly switched from fossil fuel to nuclear.

At annual growth rates in electricity demand below 3%, breeder reactors can eventually account for all nuclear power generation, although to achieve this takes at least 50 years. At higher growth rates, the ultimate contribution of breeders is limited, as discussed above.

For a given electrical output thermal reactors generally produce plutonium at least as quickly as breeders. If the introduction of breeders is delayed, plutonium stocks can, therefore, be built up, enabling breeder capacity to increase rapidly once the breeder programme begins. Even a delay of 30 years does not significantly affect the time required for the breeder to reach the maximum possible contribution, as shown in Fig. 2.

Forecasts of the rate of growth of breeder reactor capacity and the impact of uranium imports to the UK are complicated by the present over-capacity of power stations and the plutonium stocks from existing stations. Approximate estimates of uranium requirements in the first 20 years of next century are given in Table 1, assuming that an early decision to go ahead with a demonstration-scale breeder reactor is made. This would enable the first orders for commercial plants to be made by the middle/late 1980s for commissioning in 1995.

The introduction of breeder reactors will, on this timescale, only reduce uranium consumption to just under half of the requirements of a nuclear programme based on the PWR alone and will show little advantage over the CANDU thermal reactor. The choice of thermal reactor system can, therefore, have a similar impact on uranium requirements to the breeder on this timescale, and cannot be divorced from the decision to pursue breeder technology. The sixty-fold improvement in uranium utilisation frequency claimed for the breeder will not be realised for the system as a whole until well into next century. Although the medium-term savings will be worthwhile provided uranium prices are high, breeder technology cannot ensure a secure, indigenous supply of energy, effectively insulating the UK from the expense of high cost uranium, for more than half a century.

Economics of breeder reactor systems

Although it has been claimed that the capital cost of fast breeder power stations need not be greater than that of thermal stations, it now seems likely that, even if there are no major technical difficulties, the cost will be at least 25% more than that of a PWR. In order to be economically viable, this additional capital investment must be paid for by the lower fuel costs. At present, the cost of uranium ore accounts for less than 10% of the total generation cost so that, other factors being equal, a substantial increase in the price of uranium would be necessary to justify the extra capital cost of a breeder reactor.

This is confirmed by a more detailed analysis, which shows that the approximate cost of uranium ore needed for a breeder to produce electricity as cheaply as a thermal reactor is directly proportional to the additional capital cost of the breeder. (The plutonium produced by the breeder and thermal reactors is credited with its value as a supplementary fuel in thermal reactors, although in the case of the breeder this credit is more than offset—at a 10% discount rate—by the cost of the initial charge of plutonium.) Although this analysis is necessarily approximate, such a relationship means that the FBR is unlikely to give visible economic benefits until uranium prices have increased to \$100–\$200 per pound of U_3O_8 . This represents an increase in real terms of three to six times.

The rate at which uranium prices will move upwards depends on the future growth of world nuclear capacity and reserves and discoveries of high grade uranium ores. Neither of these can be estimated with any certainty and no consensus of opinion exists on the earliest date at which this consideration would be decisive in favour of the breeder. The UKAEA anticipates that uranium prices will increase to \$100–\$200 a pound when all the high grade ores are committed to existing nuclear programmes in the 1990s, and conclude that breeder capacity should be built up as quickly as possible. The Central Electricity Generating Board (CEGB), however, takes the view that the main justification for the FBR is in reducing requirements for uranium in the long term, as resources may be limited, rather than on economic grounds.

In order for nuclear energy to remain competitive for base load power generation duty at uranium prices in the range \$100 to \$200 a pound, fossil fuel prices would have to rise by between 50% and 100% relative to power station construction costs. Although, in the UK, power station coal now costs about 2½ times as much as it did three years ago (an increase even allowing

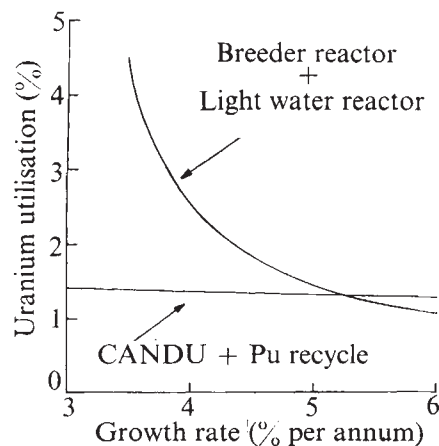


Fig. 1 Uranium utilisation in a steadily expanding system.

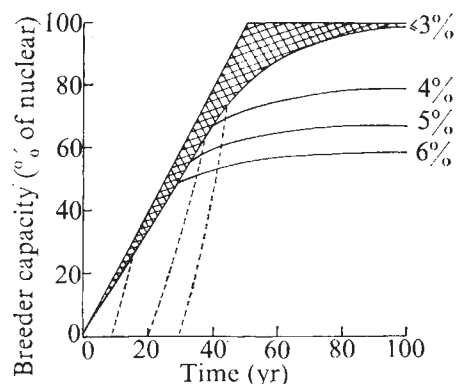


Fig. 2 Rate of growth of breeder capacity in an idealised system assuming various annual growth rates for electricity demand. For the 3% annual growth curve, the effects of delaying introduction of the fast breeder by 10, 20 and 30 years are shown by dashed lines.

for inflation), power station construction costs have risen by a similar amount over the same period, leaving the relativities in power generation costs not greatly different.

There is clearly a possibility that the increased cost of nuclear power generation brought about by high uranium prices will either reduce the competitiveness of nuclear electricity compared with other forms of energy or, should the price of the alternatives also increase, inhibit economic growth and consequently energy demand. In either case, the assumption of a continued high rate of growth in nuclear capacity after high grade uranium ores are exhausted appears open to question.

Fossil fuels

The justification for the breeder is essentially long term. No clear economic advantage is likely until uranium prices have risen three to six times above their present level. Furthermore, the contribution of a breeder/LWR system to reducing uranium requirements is unlikely to exceed that of an

efficient thermal reactor system until well into the next century.

If demand continues to grow so that it becomes impracticable to rely on thermal reactors, fossil fuels, and renewable resources for power generation, the breeder reactor would have an important contribution to make. It is against this scenario that the development of breeder technology could be considered a valuable assurance of adequate electricity supplies.

After the turn of the century, it is expected that crude oil and natural gas production will begin to decline. This, because of the unique advantages of fossil fuels, will encourage substitution either directly or indirectly, by coal. For that to occur most efficiently, new technologies for the combustion of coal and its conversion into substitute hydrocarbon fuels are required.

The development of coal conversion processes and breeder technology have certain similarities. In both cases, the construction of demonstration scale plants will soon be required. The earliest times by which each technology need be applied in the UK are similar, although both could, if required, be introduced before they become economic in order to forestall sudden increases in energy prices. Once the technology has been demonstrated,

however, the rate of commercial exploitation of coal conversion processes is not limited as for the fast breeder reactor, and a significant impact on energy supplies should be possible in a comparatively short period of time. Furthermore, the UK is already in a strong position on coal technology and the export potential of coal conversion processes should be no less than that of breeder technology.

The size of the relative contributions of breeder technology and coal conversion processes depends on whether electricity will continue to penetrate the energy market as prices increase. It is probable that substitute fuels from coal will be most effective competitors for nuclear electricity in the heating market. The estimated capital cost per unit of energy output of, for example, a plant to convert coal to substitute natural gas is about one third that of a nuclear power station, and the thermal efficiency is approximately double. Furthermore, the low load factors associated with the heating demand do not affect the economics of coal conversion as much as electricity generation. The use of nuclear energy for other than electricity generation does not look attractive at present, and would require considerable technical problems to be solved.

In conclusion, breeder reactors are capable of making a substantial contribution to energy supplies by using the uranium rejected from the thermal reactor programme. The availability of plutonium is likely to limit the rate at which breeder reactors can be built for several decades. If a vigorous development programme enabled breeder reactors to be introduced commercially by the 1990s, even although they would probably not be economic by this date, they would be unable to insulate the UK effectively from the need for expensive uranium imports until well into next century. The need to develop and demonstrate breeder technology can therefore be compared with the development of other medium-term energy options. Coal conversion processes, in particular, will be required to play a vital role in this timescale and merit no less attention and priority than breeder technology. □

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¹ Leslie, D. C., *Aspects of Energy Conversion* (edit. by Blair, I. M., Jones, B. D., and Van Horn, A. J.), (Pergamon, Oxford, 1976).

² Royal Commission on Environmental Pollution, *Sixth Report: Nuclear Power and the Environment* (HMSO, London, 1976).

³ Vaughan, R. D., and Farmer, A. A., *Proc. Instn Mech. Engrs*, 190, 30/76, 163.

⁴ CEBG Corporate Plan 1976 (CEGB, London, 1976).

Dolphin dissonance

Colin Norman reports from Washington on the threat that tuna fishing poses for dolphins

IN the past 15 years, between 5 and 6 million dolphins are believed to have drowned in the nets of tuna fishermen. The slaughter of these highly intelligent, friendly mammals has sparked one of the most bitter and complex conservation battles ever waged in the United States. A number of environmental groups, backed to some extent by the federal government, are fighting to protect the animals, while the tuna industry, according to its spokesmen, is fighting for its own survival. The battle is now focused on regulations, due to take effect in January, which conservationists say are vitally needed to protect the dolphin schools, but which the tuna industry claims will put the United States tuna fleet out of business.

The dolphin owes its plight to the fact that, for unexplained reasons, some species frequently swim with yellowfin tuna—a type of tuna which is in high demand and which fetches the highest prices. Because the air-breathing dolphins swim on the surface, they have for decades provided

tuna fishermen with a convenient guide to the location of schools of yellowfin. Until the late 1950s, tuna fishing was mostly done by hook and line, and the dolphins lost nothing by showing fishermen the way to yellowfin schools. But a technological innovation in the industry rapidly changed all that.

The innovation was the development of a massive net, called a purse seine, which tuna fishermen spread around dolphin schools and then draw in to land the yellowfin beneath the dolphins. It is a very effective way to catch tuna, but the problem is that dolphins frequently become entangled in the nets and, when they can't get to the surface to breathe, they drown. During the 1960s, an average of about 400,000 dolphins a year were dying in tuna nets; since they have no commercial value, they were thrown overboard.

Because the dolphin schools play a valuable role in leading tuna boats to yellowfin, it is in the fishermen's interest to try to preserve the animals by reducing the slaughter. They began to experiment with various manoeuvres

and changes to the nets in the 1960s, but the killing continued virtually unabated until 1972. At that point, Congress stepped in.

Public concern

Public concern over the killing of marine mammals had been aroused by the plight of many species of whales, which had been hunted virtually to extinction, and the needless slaughter of dolphins by tuna fishermen consequently became an emotionally charged issue in the early 1970s. Congress responded to the concern by passing the Marine Mammal Protection Act (MMPA) in 1972.

A compromise between the argument that the tuna industry would be wiped out by a total ban on the killing of dolphins, and the need to protect the animals, the act essentially gave the industry a two-year grace period. By October 1974, the act said, the killing of dolphins by purse seiners must be reduced to "insignificant levels approaching zero".

Congress had been led to believe that such a requirement could be met. During testimony before a House committee in 1971, Joe Medina, a tuna boat captain, reported that trials with a modified purse seine had reduced the kill of dolphin in a 'set' to insignificant amounts. "This new net has been a

salvation for us, and I think we have the problem licked", he said. Unfortunately, his optimism was unjustified.

According to a study by a panel of experts last July, the total number of dolphin killed by all tuna fishermen, including non-US operators, dropped from 348,000 in 1972 to 217,000 in 1973, and it dipped again to 120,000 in 1974. But it increased last year, reaching 181,000. Clearly, the requirements of the MMPA were not being met and the Commerce Department, which was supposed to be enforcing the Act, wasn't doing much about it.

In December 1975, the Commerce Department moved. It announced that it would permit fishing of yellowfin associated with dolphins in 1976, provided estimates made in the first quarter of the year indicated that the total 1976 dolphin kill would be less than 70% of the 1975 kill. The announcement raised a storm of protest from environmentalists, who went to court to try to force sterner measures on the industry. On May 11, 1976, Judge Richey in the Federal court in Washington DC set the tuna industry back on its heels.

In an unprecedented decision which minced few words, Judge Richey ruled that the Commerce Department's proposal violated the MMPA. The act, he said, places the interests of marine mammals above the economic interests of the tuna industry, and he ruled that all killing of dolphins by US fishermen must cease by the end of the month. The prohibition, he said, should stay in effect until the Department of Commerce can determine "that such killing is not to the disadvantage" of dolphin stocks. The decision was upheld by the Court of Appeals, but a delay in implementing it was granted until January 1, 1977.

In the meantime, the Department of Commerce came up with some new regulations for 1976. It said that tuna fishermen could continue to fish for yellowfin associated with dolphins until the total dolphin kill for the year reached 78,000. That level was reached on October 19, and after the tuna industry had exhausted its legal appeals, all killing of dolphin by US fishermen supposedly stopped in mid-November.

Conference of experts

In July, the department called a conference of experts in marine resources to try to ascertain present population levels of various dolphin species. The panel was asked to determine whether any species is in danger of falling below the so-called optimum sustainable population (OSP) level, and to try to estimate the impact on each species of various levels of kill next year.



Tuna fishermen at work

The conferees reported that the spotted dolphin population—the species most often caught in tuna nets—has been reduced to about 64% of its original level since the introduction of the purse seine. The eastern spinner dolphin—one of a number of types of dolphin which twist in the air when they jump out of the water—was reckoned to be in the worst shape; it has declined to nearly 50% of its original population level. The conferees said that a 50% decline in the population of any species would put that species below its OSP level.

One of the conferees, Dr William Aron, Director of the Office of Ecology and Environmental Conservation in the National Oceanic And Atmospheric Administration, testified last month that "while I do not contend that any of the (dolphin) stocks under consideration are presently endangered or threatened with extinction, past history indicates that a cautious approach is warranted". Aron argued that the unhappy experience with whale populations "suggests that a reduction in cetacean populations to much under half their original stock size may present a significant threat to survival of those populations".

On the basis of the conference report, the Department of Commerce issued in October regulations for 1977 which are designed to ensure that the number of animals of each species of dolphin will increase or at least remain within their OSP levels, an objective consistent with Judge Ritchie's ruling. The department essentially took the maximum kill levels which would still allow numbers of each species to increase, estimated the likely kill by non-US fleets, and set quotas for US tuna fishermen accordingly. The proposed regulations sent the tuna industry running for its lawyers and, if the industry's figures are accurate,

fighting for its survival. The industry is appealing the regulations in a lengthy public hearing.

The regulations would limit the total dolphin kill next year to some 61,400 animals, of which US fishermen would be able to kill some 29,900. In particular, the regulations would prohibit US tuna boats from setting their nets around dolphin schools containing any spinner dolphin.

Welcomes and worries

The proposed regulations have been welcomed by environmentalists. Christine Stevens, secretary of the Society for Animal Protective Legislation, calls the proposed quotas "a tremendous step in the right direction", though she points out that they are still some way from the MMPA's goal of insignificant mortality. And environmental groups, led by William Butler of the Environmental Defense Fund, have generally argued in favour of the proposals in the public hearings.

Nevertheless, the regulations will clearly have a severe economic impact on the industry. Even the Commerce Department's environmental impact statement on the proposals notes that the effect "may be severe". Unless the industry can reduce the number of dolphins killed per ton of tuna, the impact statement suggests that the US catch will drop by about one-third, which "could lead to the collapse of the US fleet if exvessel prices do not rise proportionately".

Industry spokesmen are now less restrained in their views. Frank Alverson, vice-president of Living Marine Resources, a research organisation supported by the tuna industry, claims that the total prohibition on netting spinner dolphin alone will "automatically deny the US fleet access to 68,000 tons of fish", valued at about \$45 million. That figure represents the

amount of yellowfin caught this year in association with spinner dolphin.

Environmentalists tend to take some of the industry's dire warnings with a pinch of salt, however. In particular, Butler has pointed out that tuna taken in association with dolphin actually account for a relatively small fraction of the total tuna catch. The Japanese tuna fleet, which is one of the largest producers, does not fish on dolphin at all. And a study done last year by Gordon Broadhead, president of Living Marine Resources, found that nearly 60% of the US tuna catch in 1975 consisted of skipjack or yellowfin caught when swimming in schools separate from dolphins. It is therefore argued that the US fleet will simply turn its attention to those other sources of tuna. Industry spokesmen counter, however, that such a switch would send the US fleet into areas already heavily fished by foreign vessels, and they argue that next year's skipjack catch is likely, on the basis of past trends, to be below average.

The industry therefore claims that the regulations will cause heavy losses among tuna boat operators, many of whom are already on the verge of bankruptcy. The industry is therefore fighting the regulations on several fronts. One line of attack is to challenge the estimated population levels, particularly that for the eastern spinner dolphin. Another is to argue that the regulations will penalise US tuna fishermen while allowing foreign fleets to fish at will.

Troublesome problem

The problem with non-US fleets could be troublesome. There is no mechanism available to reduce dolphin kills internationally, and thus, in meeting the

court requirement that there should be no detrimental impact on dolphin stocks, the Department of Commerce had to focus its attention entirely on the US fleet. The upshot is that if the regulations are put into effect, US fishermen will be denied access to tuna which could be available to other fleets and if the effect is to increase foreign fishing on dolphin, the net result could be disastrous.

The Department of Commerce estimates that the kill rate among foreign vessels fishing on dolphin is more than twice as high as the kill rate of US vessels. Thus, if foreign vessels move in on yellowfin schools denied to US vessels, the net effect could be to increase dolphin mortality.

There is, however, a way in which the United States government could exert leverage to prevent such a situation. The US is now the world's chief importer of tuna—domestic supply accounts of only about half US consumption. The MMPA gives the government power to insist that imported tuna is caught in accord with US regulations, and if such a requirement is enforced, a large switch in the operation of foreign fleets may be avoided.

The industry is also arguing that it has made considerable progress in reducing the mortality of dolphins per ton of tuna, and that if it is allowed to continue fishing, further progress can be anticipated. For years, tuna vessels have used a procedure known as backing down, which essentially causes part of the net to drop below the surface, enabling most of the dolphin to swim free. Another improvement, the so-called Medina panel, consists of a strip of fine mesh around the top of the net to reduce the chance that dolphins

will become entangled in the webbing, and other gear alterations are under test. According to Alverson, the tuna fleet has so far spent about \$1.7 million in gear modifications to reduce dolphin mortality.

Nevertheless, there is good reason to believe that the industry has not been as generous in funding research as it likes to suggest. It was not until December 1975, three years after Congress passed the MMPA, that the industry established a research body to study methods to reduce the dolphin kill. Known as the Porpoise Rescue Foundation, it is being funded only to the tune of \$250,000 a year. The federal government is funding at least three times that amount of research.

Though most of the studies are concentrating on gear modifications, two projects are focusing on different approaches. One, which is being tested on captive tuna in Hawaii, consists of trying to separate the tuna from dolphins by chemical attractants, and the other, which is much further from field testing, attempts to do the same thing by acoustical means.

Whatever the success of those methods in reducing dolphin mortality, it is clear that the industry will not be able to comply next year with the MMPA's goal of near zero levels. Consequently, the Department of Commerce will be hard put to justify relaxing its proposed quotas. The result, forecasts Lewis Regenstein of the Fund for Animals, is that the industry "will be breaking down the doors of Congress" to seek legislative relief. It will be a tough fight, but if the tuna fishermen get their way, environmentalists are already talking about the possibility of a boycott of tuna. □

White hope or white elephant?

What is happening at the European Molecular Biology Laboratory? Walter Gratzer looks at its first annual report

COMING at so bleak a juncture for European science, the 1975 annual report of the European Molecular Biology Laboratory (EMBL) strikes a brave and heartening note. To readers in Britain, who have seen the traditional patrons and defenders of molecular biology, the Research Councils, cringing under the lash first of Rothschild and now of the Treasury, a refreshing feature of the report is that it makes no effort to propitiate the avenging taxpayer: not once in its 50 pages is there even a token mention of heart disease, tooth decay or lower back pain.

The laboratory was conceived at the height of what Stent has called the Golden Age of molecular biology, when it was bliss to be alive, and its ten-year gestation period has seen many changes. A document published in 1966, when the project was still in the womb of time, asserted that for the undertaking to be worthwhile a multidisciplinary structure would be essential, and that all major areas of molecular biology would have to be represented; so grand a design, it suggested, would be outside the compass of any single European national institution. The laboratory would be a

major centre of scientific excellence and a nucleus for postdoctoral training, and it would nourish the university departments of Europe with a supply of the kind of young men then appearing in such profusion in America—minted at Harvard or MIT, and finished to a high radiance at NIH or Stanford. In this way Europe would regain the initiative that had by then passed to the Americans. The EMBL therefore must be large (150 scientists, 15–20 of them permanent). In contrast to CERN its object would not be to provide plant too expensive for the individual member countries to set up; in biology, the report affirmed, the plant consisted of interacting groups of scientists with outstanding and complementary talents.

Well, circumstances, as they say, alter cases and as Thurber observed, there is no safety in numbers or in anything else. The projected size and

scope appear both now to be considerably shrunk, and the report emphasises throughout the development of technical facilities sufficiently advanced to attract or generate interesting research projects. The laboratory is of course still in an early stage of growth, and is indeed a tenant in another institute in Heidelberg, pending the completion of its new building. Its Director is Sir John Kendrew, who has carried the entire project since its inception, and has recruited a predominantly young and active staff, but none of the ageing mandarins of the European molecular biology establishment.

Trendy blend

There is a trendy blend of research topics, with a strong cellular emphasis. The new investor, assembling his first portfolio, is of course best placed to take account of the steady rise in neurones and cell motility futures for example, without the pain of first getting out of slumping commodities, such as ribosomes or bacteriophage. The laboratory, then, has groups working on membranes in viruses and mitochondria, a division of biological structure (for the present confined to the development of methods in scanning electron microscopy), and a large division of cell biology, in which are subsumed four varied areas of research. There is a group working on chromatin and another on the control of chromosomal activity, using as the experimental material the giant chromosomes of insect salivary glands. There is a programme on control of morphogenesis, involving the isolation and study of peptide hormones that regulate differentiation in *Hydra*. An imaginative departure, not perhaps without the danger of creating an island within the laboratory, is the inclusion of a programme under Dr N. Strausfeld, concerned with the neuroanatomy of the visual system of insects.

In addition to the activities in its Heidelberg laboratory, the EMBL has absorbed two major existing projects which in fact seem now to constitute its most substantial undertakings. These are facilities for X-ray diffraction with high-intensity radiation from a synchrotron and for neutron diffraction, and are based respectively at Hamburg and at Grenoble. These outstations are operated as a service to European workers, and are being furnished with supporting laboratories for preparative and related work. The neutron source has already been put to widespread use, and (at an estimated cost, as I am told, of \$1 per scattered neutron) provides an example of an installation that could nowadays scarcely be run on any but an international basis. The Grenoble outstation is directed by a member of the

laboratory staff, Dr A. Miller, and is evidently a thriving concern.

The synchrotron X-ray source in Hamburg was developed and delivered into the care of the EMBL by Professor Ken Holmes, and its potential is undeniably enormous. Some spectacular exercises have already been performed, and a number of diffraction photographs of truly remarkable quality from insect muscle have made the rounds of the conferences, and have apparently sent the adrenalin coursing through the veins of fibre crystallographers. By following the evolution of a single reflection on addition of ADP to a muscle fibre, it has even proved possible to obtain a titration curve, and from it a binding constant. By making use of the larger flux from a storage ring, also in Hamburg, further advances are anticipated.

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What questions will be answered with the aid of the new diffraction technology is not yet clear. However the report suggests that time-resolved diffraction in the millisecond range may soon become a reality, and with it structural stopped-flow experiments. The system most likely to yield to such an approach is obviously the cross-bridged cycle of muscle, but it is perhaps a valid article of faith that a technique so potent will foment other interesting applications.

Sober document

A few purple passages aside, the report is a sober document that offers few hostages to fortune; it is a pity though that it has come so late and gives no financial details. Many other interesting questions are left unanswered. The report lists a scientific staff of fourteen, a large proportion of them German, and some twenty visiting workers and research fellows. The final pattern of the laboratory has therefore yet to emerge. One wonders, however, where the young postdoctoral workers who are supposed to be the immediate beneficiaries of the institution are to come from. Not presumably from America, for then the EMBL will lay itself open to the charge, often levelled (however unfairly) at the MRC's Laboratory of Molecular Biology in Cambridge: that its unique intellectual

resources have been used to train American rather than indigenous workers.

And if it is to cater for Europeans, who will provide financial patronage? The number of fellowships available in Europe can be counted on rather few fingers, if one excludes EMBO. On the other hand the laboratory would be defeating the aims of its parent body if like a growing cuckoo in the EMBO nest it were to claim an increasing proportion of each year's fellowship allocation, at the expense of the very laboratories around Europe that the fellowship programme was designed to help.

What is reassuring at all events is that the report stands by the principle of the financial independence of EMBL and EMBO. There is therefore presumably no danger that, whatever economic hardships may lie ahead, the laboratory will ever be driven to continue its planned expansion at the expense of the fellowship programme. For nothing as felicitous as the EMBO fellowship scheme has emerged to invigorate European science in many a decade. It was conceived in the clear-sighted conviction that European biological science can become greater than the sum of its parts only through expansion of the scientific community, which in turn demands the kind of fluidity that has long been one of the bases of the American success. The impact of the EMBO fellowships has already been prodigious, and the Founding Fathers of the programme must be aware that it will be remembered in their favour on the Day of Judgement.

The first annual report of the EMBL suggests that as good a start has been made as could have been hoped. There seem at the moment no grounds for fears, occasionally articulated by the churlish in the early days of the project, that the laboratory would grow into a *monstre sacré*, which would consume too much of the limited pool of funds and talent available to the university departments. Indeed, in such a time of blight, it is opportunity rather than talent that is in short supply, and the additional jobs that the laboratory is to provide will be welcome.

As to whether the EMBL will be a success at the level at which it was conceived—as the Oxford historian is supposed to have said when questioned about the effects of the French Revolution, it is too early to tell. All biologists in Europe, and for that matter America, will wish to congratulate Sir John Kendrew on getting this courageous project launched. Many will see its progress as a barometer of the prevailing intellectual climate, and all, no doubt, will wish it well. □

IN BRIEF

Nuclear committee move

Democrats in the House of Representatives last week hammered a large nail into the coffin of the Joint Committee on Atomic Energy by voting to strip the committee of its legislative power. The move, if upheld in the full house when it reconvenes in January, would break the Joint Committee's thirty-year monopoly over nuclear legislation, and it would place nuclear matters in the hands of the committees which are likely to be more critical of the nuclear programme. The vote in the House Democratic Caucus, led by Representative Jonathan Bingham, would divide the committee's legislative authority among the Science and Technology Committee, the Commerce Committee and the Interior Committee, leaving the Joint Committee as a deliberate body with no power to handle

legislation. Similar moves will be discussed in the Senate next month as part of a complete overhaul of the Senate committee system.

Environment Ministers meet

As widely expected, the Council of Environment Ministers of the European Community, meeting in Brussels last week, could not agree on Commission proposals for dealing with the discharge into the aquatic environment of waste from titanium dioxide and paper pulp plants. The difficulty, as on similar issues before, concerned whether controls should apply to emissions directly or indirectly to the environment receiving them. The Council was able to agree to a new five-year (1977-81) environment programme focusing on air, water and noise.

UK-USSR agreement

Anglo-Soviet cooperation on environmental protection is to be extended. The third meeting of the joint UK-USSR Committee on Cooperation in the Field of Environmental Protection, held earlier this month in London, approved a plan for 1977 which includes the establishment of a working group on nature conservation, the setting up of a staged programme on urban transport and the rehabilitation and maintenance of residential areas, a work programme on land reclamation, and a study of possible cooperation regarding gas cleaning and dust elimination. The prevention and elimination of oil spills at sea is to be the subject of feasibility study. Topics are selected not on a basis of absolute priorities, but according to whether they produce a valuable sharing of information.

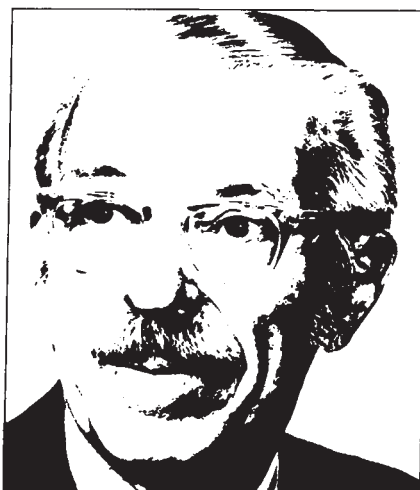
It is an old familiar story regarding industrial research that when sales decline, basic research is one of the first items to get the axe (rather than the President's plane). The justification is that accumulated knowledge is sufficient to keep things going for at least a while. In 1966, this philosophy became nationwide when President Lyndon Johnson was able to shift the federal support of science away from basic research and towards what is termed "mission-oriented research" or "research in the service of man". He said, for example, "We must make sure that no lifesaving discovery is locked up in the laboratory" (as if this were possible).

Professor Charles Tidball points out that the shift was made possible by anecdotal presentation of information to lawmakers. A factual examination of the background of "applied research" shows, however, that it is actually based largely on fundamental findings.

Decisions as to "what type" of science should be supported, or even permitted, depends increasingly on persuasive language, and today the language must be terse, so as to fit TV programmes. Slogans and phrases such as "environmental impact", "recombinant DNA", "carcinogenic chemicals", "endangered species", "noise pollution", "megavitamin therapy", and "world wildlife" are the current persuaders. They enable complicated matters to be put in neat little boxes that decision-makers can handle.

I was astonished to find recently

that one of the most popular persuaders of today, "food additives", is only eighteen years old. It did not become a category until the Food Additives Amendment of 1958. I regard the term, if you will excuse the cliché, as a pollution of the language, for most users of the phrase seem to think it means a class of substances. Actually

Language in action

THOMAS H. JUKES

it refers to a use to which diverse materials, many of them accepted foods, are put. There is a strong movement "against" food additives, and there is even an organisation "The Feingold Association", dedicated to their exorcism. The members are followers of an allergist, Dr Benjamin

Feingold, who has published a book (many MDs publish books instead of writing scientific articles) about hyperkinesis in children, and its relief, and that of their parents, by a diet containing no "food additives". I have not seen in any writings supportive of the "Feingold treatment" mention of the fact that numerous "natural ingredients" of food are more toxic than additives at levels of permitted usage.

It may be that Dr Feingold has found a way to rid harassed and inactive parents of that nuisance, the "hyperactive child", which, incidentally, is another slogan. But consider a partial list of what must be eliminated from intake or contact: apples, blackberries, cherries, peaches, cucumbers, pickles, tomatoes, ice cream, bakery goods (except "plain bread"), luncheon meats, jam or jelly, gin and all distilled drinks except vodka, tea, beer, wine vinegar, all soft drinks, aspirin, perfumes, toothpaste and toothpowder.

Is it possible that the goodies in this incredibly diverse list share any common biochemical or pharmacodynamic properties that make each and all of them inductive of "hyperactivity"? I find this postulation incredible, but millions of dollars will be spent to seek out the answer. I suggest that this expenditure is yet another example of "language in action".

Perhaps, however, sluggish adults should help themselves to tomatoes, ice cream, gin, toothpaste and perfume to induce hyperactivity.

news and views

Controversy over the extragalactic distance scale

from M. Rowan-Robinson

THE redshift-distance relation for galaxies discovered by Hubble and Lundmark in the 1920s is the most tangible evidence we have for an expanding universe. The constant of proportionality, the Hubble constant, can be interpreted as the expansion time-scale of the universe. Yet in spite of the consumption of many thousands of hours of telescope time, the extragalactic distance scale remains a subject of controversy, highlighted by a disappointingly inconclusive International Astronomical Union symposium at Paris in September.

At the symposium Tammann summarised the massive programme of galaxy distance measurements that he and Sandage have been pursuing, culminating in a value for the Hubble constant, H_0 , of around 50 km s^{-1} per Mpc (for a recent review see *Nature*, **262**, 97; 1976). Yet almost all other workers reporting at the symposium disagreed with the Sandage and Tammann distance scale, preferring a value for H_0 closer to 80. For example, van den Bergh and de Vaucouleurs reported significantly lower distances for members of the Local Group of galaxies, to which our Milky Way galaxy belongs. Hanes' work on globular clusters in galaxies in the Virgo cluster, the nearest of the great clusters of galaxies that determine the Hubble relation on the large scale, led to a distance estimate for Virgo of 12.5 Mpc (1 Mpc = 3.26 million light years) instead of the Sandage and Tammann value of 22. And Tully and Fisher used their 21-cm neutral hydrogen line method to obtain a value for H_0 of 75.

It is therefore fascinating to see in the latest *Astrophysical Journal* (**210**, 7; 1976) Sandage and Tammann's version of this last method. They take Tully and Fisher's data, reanalyse and recalibrate it, and emerge with a Hubble constant of 50.3 ± 4.2 . The 21-cm method depends on a correlation found by Tully and Fisher between the 21-cm hydrogen line

width and the optical luminosity of a spiral galaxy (one has to have some galaxies with distances known more directly to calibrate this relation). The apparent optical brightness of a galaxy then gives the distance through the inverse square law.

Sandage and Tammann use the method to obtain the distance of the Virgo cluster, and compare the result with six other methods (including, ironically, an earlier estimate by Hanes which agreed with theirs). The adopted Virgo distance, 22 Mpc, is then combined in three different ways with the mean velocity of the cluster

to yield $H_0 = 50.3 \pm 7.0$, 50.3 ± 4.2 , 50.3 ± 4.2 . The authors remark "That the values of H_0 from all three methods are the same is clearly fortuitous". If the agreement between these three means at so high a significance level is indeed simply good luck, then heaven protect these authors from bad luck.

Do these disagreements over H_0 , which appear even when the same data is used, matter? Well, first, the actual value of H_0 matters to cosmologists, because by comparing it with the age of our own and other galaxies they can deduce how long

Precise Lamb shift interval

from Peter Knight

FOR two years now, we have had two slightly different theoretical values for the hydrogenic $n=2$ Lamb shift interval. The more recent value, due to P. J. Mohr (*Phys. Rev. Lett.*, **34**, 1050; 1975) is 1057.864 ± 0.014 MHz, and should be compared with the earlier value of 1057.912 ± 0.011 MHz due to G. W. Erickson (*Phys. Rev. Lett.*, **27**, 780; 1971). Published experimental values up to 1975 lacked the ultra-high resolution needed to support one value rather than the other. The 100 MHz natural linewidth of the Lamb transition imposes severe limitations on precision experiments, and great care is needed in the lineshape analysis.

At Harvard, S. R. Lundeen and F. M. Pipkin (*Phys. Rev. Lett.*, **34**, 1368; 1975) used a Ramsey-type separated-oscillatory-field technique combined with a fast atomic beam to achieve resonance widths of one-third the natural width, and an improvement of a factor of 3 in the precision of the experimental value for the $n=2$ hydrogenic Lamb shift, obtaining a value of 1057.893 ± 0.020 MHz. Unfortunately this still overlaps with and is consistent with both theoretical values. This situation and future prospects were

recently reviewed by Pipkin (*Comments Atom. molec. Phys.*, **5**, 45; 1975).

More recently D. Andrews and G. Newton at the University of Sussex report the results of a new high-precision single-loop experiment, which whilst consistent with the Harvard experiment, manages to discriminate in favour of the new value of Mohr. The Sussex experimental value is 1057.862 ± 0.020 (*Phys. Rev. Lett.*, **37**, 1254; 1976).

It is unlikely that the precision of the Sussex technique can be improved significantly, since (as they say) they are at the limits of available microwave technology. Similarly the lineshape analysis of the separated-field approach is complex. One is, after all, trying to resolve a line centre to better than a few parts per million, when the fundamental natural width is as large as one-tenth of the resonance frequency. Opportunity for further progress seems to rest with the theoreticians. The source of the discrepancy between Mohr's and Erickson's theoretical values is unknown, but presumably is connected with the different ways of computing the self-energy contribution to the Lamb shift.

galaxies took to form. The big bang picture would be in trouble if the expansion time was less than the age of our Galaxy. Second, discrepancies which are such a large multiple of the quoted errors do not enhance the status of observational astronomy. At the moment the onus seems to be on the opponents of the Sandage and Tammann scale to assemble a similar body of observational data. Theoreticians will have to maintain the scepticism about H_0 that the wiser among them have always shown in the past. □

RNA and generation of positional information

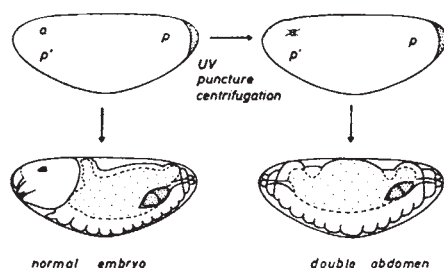
from Peter Lawrence

MOST biochemical investigation on developing systems is just conventional biochemistry on a system which happens to be developing, and is not relevant to the understanding of development in terms of the controlling molecules involved. (After all, even developing animals have to make proteins, send acids scooting round the Krebs cycle and so on). Conversely, specifically developmental phenomena, such as regulation are difficult to approach from a biochemical point of view. But several insects, including the tiny dipteran midge *Smittia*, undergo what Sander (*Wilhelm Roux Archiv.*, **167**, 336; 1971) has called "negative regulation". An embryo regulates by reacting to experimental damage, such as removal of tissue, by respecification of cells so that normal development is restored. In negative regulation, however, respecification proceeds abnormally so that a monster develops. These striking changes in *Smittia* have provided investigators with a handle on the molecular mechanisms which may be involved in specifying that pattern.

Normally the insect consists of a number of segments arranged in order from head to tail; each segment is uniquely patterned. The segment order in most insect eggs becomes defined at blastoderm (when the embryo is just a hollow ball of cells) and depends only on the positions of the cells forming at the surface of the egg, not on the lineage of their nuclei. There is thus positional information in the egg. Experiments on other insects (see Sander, *Ciba Foundation Symp.*, **29**, 241; 1975) suggest that the positional information is in the form of a gradient which is made by an interaction between two determinants, an anterior and a posterior one, at the poles of the egg. Kalthoff working in Sander's

laboratory (Kalthoff, *Roux. Arch.*, **168**, 63; 1971) discovered that local ultraviolet irradiation of the anterior pole of cleaving *Smittia* eggs (the nuclei were untouched by irradiation) caused up to 100% of the embryos to change their segment pattern from 123456789 to 98766789 (Fig. 1). This aberrant pattern is called a 'double abdomen'. The targets to ultraviolet were localised in the egg cytoplasm and distributed unevenly in the anterior third of the egg, becoming most concentrated at the anterior tip. The damage done by ultraviolet was not violent: nuclei subsequently entered the irradiated region and developed normally into cells. Only the positional information, and hence the segment pattern, seemed to be altered. Kalthoff found the ultraviolet damage could be repaired by visible light so that normal embryonic development was restored; this suggested that nucleic acid might be the target. Action spectra (Kalthoff, *Photochem. Photobiol.*, **18**, 355; 1973) showed peaks in both the protein and the nucleic acid regions.

This line has now been taken one step further by Kandler-Singer and Kalthoff (*Proc. natn. Acad. Sci.*, **73**, 3739; 1976). They were able to produce double abdomens by gently puncturing the anterior pole of the egg in water containing RNase ($0.8 \mu\text{g ml}^{-1}$). Although about half the embryos failed to develop at all, 47% of those which did made double abdomens. In water only some 5% failed to develop and none grew up with double abdomens. With an elegant control they demonstrated that it was actually the RNase activity which was responsible for altering the segment pattern. They treated the eggs with two fragments of RNase each of which is inactive separately (RNase S (active) is split by TCA fractionation into S-peptide (inactive) and S-protein (hardly active)). When solutions of the two fragments are mixed RNase activity returns. The percentage of double abdomens was nil with S-peptide, 1 with the S protein



Diagrammatic representation of the induction of a double abdomen in *Smittia* eggs by ultraviolet irradiation of the anterior pole region, puncture at the anterior pole or centrifugation with the long egg axis parallel to the centrifugal force. (From *Insect Development*, edited by Lawrence, P., Blackwells Scientific, Oxford and London, 1976.)

and about 40 both with complete RNase S and with the two fragments dissolved together. Puncturing of the eggs elsewhere than at the anterior pole produced no double abdomens. Double abdomens were formed only when the experiment was performed up to the blastoderm stage of embryogenesis. These results together with those mentioned earlier, strongly suggest that an RNA moiety is an essential component in the generation of positional information in the egg. Kandler-Singer and Kalthoff suggest it might be mRNA that is translated during cleavage.

The next stage in this investigation will ideally be the development of a positive assay in which the damage done by ultraviolet will be repaired by the injection of extracts into the anterior pole of the egg. This could lead to the first isolation of a molecule specifically involved in the generation of positional information and perhaps to some inkling as to what gradients are in molecular terms. □

Structural gene for sex pilin

from J. P. Beard

A RECENT paper from Charles Brinton's laboratory in Pittsburgh reports on an important aspect of the mechanism whereby 'male' Gram-negative bacteria are able to transfer genetic material to 'female' bacteria by conjugation. This subject has been of great interest to microbiologists for many years, since not only does the conjugation process provide a useful research tool for probing the genetic composition of *Escherichia coli* and related species, but it is also usually mediated by genes carried on plasmids in the male or donor cell. Plasmids are small extrachromosomal pieces of DNA, carried by a wide variety of bacterial genera, which are capable of autonomous replication in the host cell, and which can often transfer both themselves and chromosomal DNA in the conjugation process. Plasmids are also important as vectors of multiple antibiotic resistance genes, or of genes which confer other important properties on the host such as colicinogeny or enterotoxin production (see, for example, the *Ciba Foundation Symposium on Bacterial Episomes and Plasmids*, J. and A. Churchill, 1969), and have recently taken on a new lease of life as vectors for "genetic engineering", currently the subject of intense public discussion.

Studies of one such plasmid, the sex factor F (Hayes, *The Genetics of Bacteria and their Viruses*, Blackwell,

1968), have shown that male, or F^+ , cells produce filamentous appendages on their surfaces—sex pili, which are required for both conjugation and sensitivity to male-specific bacteriophages. In addition, it has been demonstrated that sex pili are composed of a single protein, sex pilin, of molecular weight 11,400, which contains one mol of glucose and two mol of phosphate per mol of pilin, while genetic studies have shown that the transfer process is controlled by at least twelve genes on the plasmid whose order has been established by deletion mapping to be

*traJ traA traL traE traK traB
traC traF traH traG traD traI*

It has been shown that the product of the first of these genes, *traJ*, is required for the expression of the other genes, *traA* to *traI*, which constitute a single operon (Willets, In *Microbial Drug Resistance*, edit. by Mitsuhashi and Hashimoto, University Park Press, Baltimore, 1975; and Helmuth and Achtman, *Nature*, **257**, 652; 1975). Mutants in any of the first nine genes (*J*, *A*, *L*, *E*, *K*, *B*, *C*, *F* and *H*), and some in the tenth (*traG*), lack sex pili, and complementation analysis of a range of the mutants in the presence of another *F*-like plasmid (R100-1), which specifies slightly different sex pili, suggested that *traA* was a likely candidate for the *F* pilin structural gene (Willets, *Nature new Biol.*, **230**, 183; 1971). This has now been confirmed in a series of elegant experiments by Brinton's group (Minkley, Polen, Brinton and Ippen-Ihler, *J. molec. Biol.*, **108**, 111; 1976) in which they took advantage of the fact that sex pilin contains only two tyrosine residues per molecule. By using amber (nonsense) mutants in the *traA* gene which were originally isolated by Achtman *et al.* (*J. Bact.*, **106**, 529; 1971) and a *supF* host strain which suppresses amber mutations by inserting a tyrosine residue at the amber site during protein synthesis, Minkley *et al.* have been able to isolate the sex pili specified by the mutant plasmid in the suppressing host. Subsequent iodination of the pilin protein derived from these sex pili, using radioactive iodine-125, and preparation of peptide maps (2-dimensional separation on thin layers of cellulose) after tryptic digestion, has revealed three radioactive spots in the case of tyrosine-suppressed *traA* sex pilin compared with two radioactive spots from pilin obtained from serine-suppressed *traA* sex pili (produced by a *supD* host carrying the *traA* mutant plasmid).

This neat identification of *traA* as the structural gene for sex pilin is a first step towards a detailed biochemical analysis of the biosynthesis of sex pili, and it should form the basis of a clearer understanding of the roles of the re-

maining *tra* genes in the processes of conjugation and male-specific phage infection. In the long term, this work could provide molecular biologists with a unique model for study of a complex surface polymer, specified by an easily-manipulated plasmid genome, whose biosynthesis and assembly is likely to involve important interactions with other, host-specified surface components of the bacterial cell. □

Watchers of the skies

from David W. Hughes

NETWORKS of all-sky cameras are coming and going in modern astronomical research. Coming because the Russians are just installing an enormous new system in the south of the USSR. Going because, almost simultaneously, the American Smithsonian Institution is abandoning and dismantling its Prairie network.

Zotkin, Simonenko, Fedynsky and Khotinok (Meteorite Committee of the USSR Academy of Science) and Kramer (Astronomical Observatory of the Odessa University) discuss the Russian Network in a recent paper in *Meteoritika* (USSR Acad. Press, **35**, 3; 1976). This will have 39 camera stations separated by 180 to 240 km spaced throughout 900,000 square km of the River Don basin, the Ukrainian and Moldavian Republics and the North Caucasus. This is a relatively flat, densely populated part of the country with well-developed communication links. It is hoped that the network will provide about 100 photographs of fireballs brighter than -9.0 visual magnitude over a 5-year period. The velocity, time of appearance, atmospheric trajectory and previous orbit of each of the fireball-producing meteoroids can be obtained by careful analysis of the photographic plates. It is estimated that between 1 and 3 of the fireballs photographed will also be so bright that the causative interplanetary body will not completely ablate in the atmosphere and a part of it will fall to ground as a meteorite. The fireball's atmospheric trajectory enables the area of fall to be calculated and it is hoped that a detailed search of this region will lead to the recovery of the meteorite. This will not only increase the number of meteorites available for analysis but much more importantly will provide a special class of meteorites which have well-known orbits. Only three meteorites, Pribram, Lost City and Farmington are in this special category so far.

The Russian system will be the fourth in Europe. The first one to be set up was over the whole of Czechoslovakia (*Bull Astr. Inst. Czech.*, **16**, 15; 1965)

which has lately been joined by a similar network covering the south of the Federal Republic of Germany. There is also a large network in the British Isles run by the Meteor Section of the British Astronomical Association (*J. Br. Astr. Assoc.*, **85**, 150; 1975). In North America a Canadian system watches over southern Alberta, Saskatchewan and Manitoba. The old Prairie network (*SAO Special Report* No. 193, 1965) used to cover all of South Dakota, Nebraska, Kansas, Oklahoma, Mississippi, Iowa and Illinois. It was hoped that this Prairie network would lead to the recovery of at least one meteorite per year but unfortunately this was not to be. Many fireballs were photographed and our knowledge of these awe-inspiring objects increased enormously as a result of the data collected but paradoxically it was found that few brilliant fireballs actually lead to meteorite falls. The Lost City meteorite was the only one found during all the years of operation.

A second use of these networks has recently come to light. At the Ondřejov Observatory near Prague, Czechoslovakia, an Opton-Distagon fish eye objective on a large (9×12 cm) plate camera is used to photograph systematically the entire celestial hemisphere each moonless clear night. This has been done since March 1975 when a programme was started to replace the old 36 cm convex mirror-type all sky cameras previously used throughout the Czechoslovak network by these newer cameras. The image quality produced by this fish-eye lens is excellent. The camera is driven and with the usual 4 h exposure, reaches a magnitude limit of +11 and has a resolution of 1 min arc. The changes in image quality as far as 70° from the centre of the field of view is still small enough to be removable by small correction terms. Bocek, Cepelch, Ježková and Novák used the camera to take a set of 13 photographs of Nova Cygni 1975. The image definition was so good that there was an almost linear dependence of star image diameter on stellar magnitude. Over 20 comparison stars could be used on each plate, all at a zenith distance of less than 40° and with comparative ease the visual magnitude (*V*) of Nova Cygni could be obtained to a precision of ± 0.10 magnitudes. These observations, made between August 31.8 and September 2.9, are reported in the latest edition of the *Bulletin of the Astronomical Institutes of Czechoslovakia* (**27**, 190; 1976).

With the ever increasing use of these sophisticated cameras throughout Czechoslovakia it is hoped that, as well as leading to meteorite finds, the

rise in brightness of new novae can be accurately plotted as well as the decay. With a limiting magnitude of +11 it is hoped that many more than the 2.2 novae normally discovered each year will be found. □

Probing specific translational controls

from Martin Blundell

In principle, the expression of genetic information may be regulated either at transcription or at translation. Control of transcription eliminates the synthesis of unnecessary messenger RNA but control of translation could act more quickly, particularly in eukaryotic cells, where messenger half-lives are measured in hours.

Most control seems to be exerted at transcription or possibly (in eukaryotes) during the processing and transport of mRNA from the nucleus. Nevertheless, translational control is clearly established in eukaryotic cells. In reticulocytes, for example, the translation of globin and other mRNAs is controlled by diverse agents such as hemin and double-stranded RNA. Other cells respond in the same way to virus infection and alteration of their growth medium. In general, this sort of translational control appears rather unspecific. Thus, haem deficiency shuts off all reticulocyte protein synthesis, not just the synthesis of globins.

Translational controls seem more difficult to detect in *E. coli*, where message half-lives are much shorter (even when expressed as a fraction of the doubling time of the cell). Translational controls do exist, however, and they may be more specific than those in eukaryotes.

When *E. coli* is infected by phage T7, a T7 gene makes a translational repressor which switches off host protein synthesis (Herrlich *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1088; 1974) presumably to maximise production of T7 proteins. Another kind of translational control is seen during infection by phage λ (Ray and Pearson, *J. molec. Biol.*, **85**, 163; 1975; *Nature*, **263**, 647; 1976). The late genes of λ are all transcribed at about the same rate, but the number of protein molecules made from different genes varies nearly a thousand-fold. Since the functional lifetimes of the various messages are all about 2½ min, the differences in quantity of protein observed can only be explained by varied efficiencies in translation or possibly by differences in the stability of the proteins themselves.

In uninfected *E. coli* there seems to be a control of translation of some inducible messages. In three rather different cases, the translation of β -galactosidase message is impaired when general protein synthesis appears normal. Ennis and Kievet (J. *biol. Chem.*, **251**, 2854; 1976) have studied cells recovering from inhibition of protein synthesis by either chloramphenicol or deficiency of potassium ions (the latter avoids difficulties which might arise from residual antibiotic). In these cells, *lac* mRNA is made but is only poorly translated. Other proteins as large as β -galactosidase and larger are being made, so the effect is not just a lesion in protein synthesis. The message can function *in vitro* to make the amino-terminal part of β -galactosidase, but not even this part is made *in vivo*. Although more complex explanations remain possible, the simplest is that some control on translation is operating in the recovering cells. The same authors also report under-translation of *lac* mRNA in *lac* O^c mutants and *trp-lac* fusion strains (J. *Bact.*, **125**, 1220; 1976).

The most recent work of Herrlich and Schweiger (*Proc. natn. Acad. Sci. U.S.A.*, **73**, 3386; 1976) suggests translational control of a number of inducible genes. The main thrust of their paper is to determine the mode of action of nitrofurans, a class of synthetic antibiotics in widespread use as food additives and in medicine. When low concentrations of nitrofurans are added to cultures, the synthesis of inducible proteins (β -galactosidase, tryptophanase, galactokinase and others) is inhibited while other proteins are unaffected. The possibility that this effect is mediated by cyclic AMP and catabolite activator protein is eliminated.

Three lines of evidence indicate that the drug acts on translation. First, in EDTA-permeabilised cells, nitrofurans act on β -galactosidase synthesis more rapidly than do rifampicin or actinomycin. Second, in strains where *lac* transcription is fused to nitrofuran-resistant genes (either the *trp* operon or *lac i^a*) β -galactosidase synthesis remains nitrofuran sensitive. However, when a deletion fuses the peptides made by the *lac i* and *lac z* (β -galactosidase) genes, the production of the fusion peptide is nitrofuran resistant. Third, *in vitro* synthesis of galactokinase is equally sensitive to nitrofuran whether directed by DNA or RNA, whereas *gal* transcription is unaffected.

If nitrofurans are to act specifically on the translation of certain proteins, there must be some recognition signal. The only stage at which recognition could easily occur is at the initiation of translation. This conclusion is supported by the nitrofuran-resistant

synthesis of the *lac* repressor: β -galactosidase fusion peptide and by a further experiment on galactokinase synthesis *in vitro*. When preformed polysomes are used for galactokinase synthesis, so that ribosomes have already initiated, nitrofuran no longer inhibits. Apparently a group of messengers carries a signal different from other messengers, and the translational machinery is able to recognise this signal. Such signals could presumably have evolved to provide specificity in a translational control system.

How much use does *E. coli* make of translational control? Unless a message is completely inactivated, translational control would generate polysomes with fewer ribosomes than usual, but electron micrographs (for example, *Cold Spring Harbor Symp. quant. Biol.*, Miller, **35**, 505; 1970) show that the density of ribosomes on mRNA is remarkably uniform. □

Plumes in an expanding Earth

from Peter J. Smith

THE expanding Earth hypothesis has been part of the geophysical backdrop for at least 20 years. Indeed, its origin in physics may be traced back even further, to Dirac (*Nature*, **139**, 323; 1937) who first proposed that the gravitational constant may not be a true constant but may be slowly decreasing with time. More recently, Steiner (*J. Geol. Soc. Aust.*, **14**, 99; 1967) has suggested that the gravitational constant may be varying cyclically rather than decreasing monotonically, but neither this idea nor Dirac's has received convincing proof. The point is, however, that during a period in which the gravitational constant is decreasing, the forces between particles would also be decreasing, and the volume of the Earth would therefore be increasing.

On the whole, Earth expansion has found little favour among geologists and geophysicists. It has a few very strong supporters, notably Carey (*Earth Sci. Rev.*, **11**, 105; 1975); and various workers have from time to time tried to test the hypothesis by, for example, palaeomagnetism (which can be used in theory to detect changes in the Earth's radius) and the fitting of continents onto globes with reduced radii. But such attempts have never been entirely successful, often because the data available were insufficiently accurate to resolve expansion of the Earth at low rates. The real reason for the expansion hypothesis's lack of success, however, is less the evidence against it than the

fact that it seems to be unnecessary. In other words, few are convinced that there are any major phenomena that cannot be explained by plate tectonics.

But this is far from saying that the expansion has not occurred. The question remains unresolved; and it is still open to anyone to test the hypothesis against any appropriate data. This is precisely what Stewart (*Geophys. J.*, **46**, 505; 1976) has now done using hot spots and mantle plumes. Of course, mantle plumes are as controversial as the expanding Earth. There are certainly hot spots at the Earth's surface, but whether they reflect underlying crustal-upper mantle phenomena or whether they are due to thin columns rising from the core-mantle interface is still a matter of debate.

One of the main attractions of mantle plumes, however, is the possibility that they might be laterally stable and thus provide a frame of reference fixed to the mantle. Evidence on this point is mixed, with some workers claiming to have shown that plumes remain fixed with respect to each other and others claiming to have detected relative motion. But as Stewart points out, all such exercises have been carried out assuming the Earth to have maintained a constant radius. So how do the data appear against the background of an expanding Earth?

To find out, Stewart has determined the great circle angular distances between the members of pairs of hot spots for three times—the present, 50 Myr ago and 120 Myr ago—assuming a constant terrestrial radius. For points within the same plate, the average ratio of the 50 Myr angles to the present angles is 0.98 ± 0.04 which is not significantly different from unity. The average ratio of the 120 Myr angles to the present angles, however, is 0.88 ± 0.03 . The errors on these figures are estimated to be up to 5%, largely because of the difficulty of locating accurately the centres of hot spots which may be 150 km or more across. But taken at face value the older figure suggests that the average great circle separation between members of hot spot pairs could have increased by 11–17% over the past 120 Myr and even up to 6% over the past 50 Myr.

One explanation of these remarkable results is that the hot spots are moving with respect to each other at relative speeds of $0.5\text{--}2.0\text{ cm y}^{-1}$ across the surface of an Earth with constant radius. But in that case it would be strange that the members of most pairs are moving away from each other, for in a random system one would perhaps expect as many hot spots to be converging as diverging. On the basis of Stewart's evidence, therefore, it seems much more likely that the geocentric angles between the supposed mantle

plumes remain roughly constant whilst the Earth expands to produce divergence of the hot spot locations at the surface.

As Stewart admits, his results can hardly be said to prove that the Earth has expanded. But they are a small step in that direction. □

Balancing the nutrient budget

from Peter D. Moore

THERE have been many commendable attempts to reduce to quantitative terms the influence of catastrophes on the flow of energy through and the cycle of nutrients within ecosystems. Some of the most carefully monitored experiments have come from the Hubbard Brook ecosystem studies in New Hampshire (for example, Likens *et al.*, *Ecol. Monogr.*, **40**, 23; 1970) and the Coweeta catchment area studies in North Carolina (for example, Swank and Douglas, *Science*, **185**, 857; 1974). In both these experimental areas, deciduous forest cover has been destroyed and the resultant changes in runoff water and nutrient losses have been observed. At neither of these sites, however, have the consequences of these hydrological and chemical changes been considered from the point of view of those ecosystems which receive the effluent generated by the catastrophe.

A study has recently been undertaken in Minnesota by Wright (*Ecology*, **57**, 649; 1976) in which the chemical impact of forest fire upon the nutrient budget of receptor lakes has been determined. The forested catchments of Meander Lake and Lamb Lake in the north-east of Minnesota were extensively burned in a serious fire in 1971. Changes in the nutrient budget and hydrology of these lakes were compared with those of Dogfish Lake, a similar site whose catchment lay outside the burned area.

The percentage increase in runoff attributable to the fire was 30% at Lamb Lake and 60% at Meander Lake. The latter value is considerably larger than values obtained after clear felling catchment areas at Coweeta and Hubbard Brook (15% and 40% increases respectively) and the reason for this may be the loss of the absorptive moss and humus layers after the fire in Minnesota. As it moves through the system, water may receive and may also lose various ions. On arrival at the ground, precipitation may move for a short distance over the surface, particularly if the soil is frozen. In burnt areas this water becomes enriched with phosphates. On percolating into the soil

The ecology of dragons: a reply

I AM glad that Robert May thought my article (Hogarth, *Bull. Brit. Ecol. Soc.*, **7**, 2; 1976; May, *Nature*, **264**, 16; 1976) seminal; I must, nevertheless, disagree with him on one of his criticisms. In classifying the wyvern and cockatrice separately from the dragon, he attaches more significance to limb number than to other characteristics which these three types held in common. In particular, I refer to their supernatural qualities which seem to me to be of sufficient importance to justify setting them apart in a taxonomic category separate from that of the vertebrates. Four legs do not make a tetrapod; it is no more logical to include wyverns and cockatrices with conventional vertebrates than to classify a 12-legged dragon as a myriapod. Convergent evolution has indeed occurred to a remarkable extent, but between dragons and their allies on the one hand and vertebrates on the other: not, as May suggests, between vertebrates (including the wyvern and cockatrice) and dragons.

My estimate of 5,000 years BP for the emergence of dragons is therefore feasible. The total dependence on human imagination for their existence (a unique ecological relationship) precludes an origin earlier than, say, the late Pleistocene. The absence of convincing representations of dragons in upper Palaeolithic art, and their frequent occurrence in literature and art from the time of Babylon onwards, indicates the chronological limits on the possible time of emergence. Between these limits, 5,000 years ago seems a reasonable, albeit imprecise, estimate.

P. J. HOGARTH

these phosphates are adsorbed onto soil particles, but the general cationic load of the water is increased. Stream water is considered to be equivalent chemically to this subsurface flow of water in the soil.

Wright determined nutrient budgets of phosphorus and various cations by monitoring input from precipitation and output in streamflow. The difference between these two rates is equivalent to the weathering input less any change in storage capacity in the system (including biomass growth). It is regrettable, though understandable, that these two components in the budget cannot be separated, but considerable additional measurements

would be necessary in order to achieve this. In the absence of such information, however, conclusions should be tempered with caution. For example, Wright states that nutrient cycling in the undisturbed forest is efficient, with only minor amounts leaking from the system. At the control site, Dogfish Lake, $13.7 \text{ mg m}^{-2} \text{ yr}^{-1}$ P arrives in precipitation and only 1.5 leaves in runoff. Rather than invoke the terms 'efficiency' or 'conservation' in such a situation, one should suspect an increasing storage capacity, probably due to biomass growth. If there were no storage change then inputs should be equalled by outputs, so the terms 'efficiency' and 'conservation' are not particularly informative in nutrient budget studies.

It is evident from Wright's data, however, that the output of nutrients at the burned sites exceeded expected values based on extrapolation from the control data. Such figures for increased discharge as 26% for Ca, 29% for Mg, 265% for K, 65% for Na and 93% for P were obtained from the Meander Lake catchment. Thus water entering the lakes had been substantially enriched in both phosphorus and cations.

Input of nutrients to the lakes is derived from runoff water and also directly from precipitation. At his control site Wright found that runoff was the major source of cations, but that precipitation accounted for over 80%

of the phosphorus input. This is presumably due to the adsorptive properties of the soils for phosphorus. At Meander Lake, after catchment burning, runoff inputs were increased yet further for the cations, and for phosphorus were brought up to the same level as that derived from precipitation. The total increase in phosphorus load at Meander Lake as a result of this change was 38%. In Wright's opinion this lies within the kind of year to year variation expected in phosphorus supply and does not represent a major eutrophication episode. If this is so, then the adsorption of phosphorus in the soil of the catchment is critical for the maintenance of stability of lakes in periods of catchment disturbance. □

Man's influence not yet felt by climate

from John Gribbin

THE message conveyed by Professor B. J. Mason, Director-General of the UK Meteorological Office, in a recent lecture was—don't panic. The theme of Mason's lecture (given to the Royal Society of Arts on December 1) was "Man's Influence on Weather and Climate", and his conclusion was that the climatic system is so robust, and contains so much inherent stability through the presence of negative feedback mechanisms, that man has still a long way to go before his influence becomes great enough to cause serious disruption to the natural climatic system.

Mason began his discussion by setting the influence of man in the context of the natural climatic changes that occur continually, pointing in particular to fluctuations of temperature in the United Kingdom during the three centuries for which instrumental records are available. In the past, fluctuations on the scale of 1°C variation in the annual mean temperature have had less impact on society than similar changes may have in the future; as Mason stressed, the present large world population and inadequate food reserves now put the agricultural system under unprecedented strain. "There is no question that climate is variable and that variations have a greater social and economic impact than ever before", so that changes of 1°C in the long term mean cannot be ignored—such a variation in the United Kingdom could, for example, change the length of the growing season by three weeks. But is man's influence yet approaching even this level?

Three anthropogenic influences have been widely cited as potential causes of climatic disaster: the "greenhouse

effect" caused by CO_2 from burning fossil fuels; cooling produced by dust in the atmosphere; and the effects of chlorofluorocarbons and supersonic aircraft on the stratospheric ozone layer. Mason's analysis of the problems hinged upon the computer modelling of the atmosphere which is the forte of the Met Office, and the numbers he produced were surprising, if reassuring, to at least some members of his audience. A dust layer in the stratosphere thick enough to cut off 4% of incident solar radiation, for example, warms the upper atmosphere by as much as $10\text{--}20^\circ \text{C}$, but produces no detectable effect on the troposphere in the models. And this conclusion may be borne out by events in the real atmosphere after the volcanic eruption in Bali in 1962, when the temperature of the stratosphere increased by some 6°C without any observable effects, according to Mason, on the lower atmosphere.

The ozone problem has been highlighted by controversy surrounding Concorde and spray cans, widely reported in *Nature*. Both the Met Office and American models have now shown that even a fleet of 500 SSTs flying for 5 hours per day each would not reduce the ozone content of the stratosphere by more than 1%, and although the hazard from spray can propellants seems greater, even their release of "freons" could continue at the present 700 kt yr^{-1} until 2100 AD before ozone would be reduced by 8%. Mason feels that the decision in the US to ban such propellants is over hasty, not least because if release at the present rate continued for another 5 years the climate modellers would have enough hard figures to produce better models of what is going on.

But the can is now being banned, at least in the US, and we are left with the release of CO_2 as man's only real, quantifiable contribution to climatic change yet or in the near future. Having cautioned that if the effect is real, something must have offset it in recent decades, since the Earth has actually been cooling down, not warming up, Mason suggested that within 50–100 years the effects of CO_2 release into the atmosphere will produce a warming of $1\text{--}2^\circ \text{C}$, well into the range significant for our present global society. Computer modelling indicates that rainfall patterns would be shifted by 5–10%, but the models are not yet sophisticated enough to detail the effects on specific regions of the globe. There is, says Mason, a real and significant effect here which must be investigated further with bigger and better models—but there is time to develop the necessary models and no need for panic induced by the prophets of doom. □



A hundred years ago

A NEW STAR IN CYGNUS. — On November 24, at 5h. 41m. P.M., the director of the Observatory at Athens, Prof. Schmidt, remarked a star of the third magnitude not far from ρ Cygni, which was not visible on November 20, the last clear evening previous. Its position from observations with the refractor was found to be in R.A. 21h. 36m. 50.5s., N.P.D. $47^\circ 40' 34''$ for the beginning of the present year. At midnight its light was more intense than that of η Pegasi, which is rated a third magnitude by Argelander, and very yellow.

Direct intimation of this discovery was given by Prof. Schmidt to M. Leverrier, and the Paris *Bulletin International* of December 6 contains the few particulars concerning this star which the generally unfavourable weather up to that date had permitted to be put upon record. M. Paul Henry estimated it of the fifth magnitude, so that as in the cases of the similar suddenly-visible stars of 1848 and 1866, it would appear to have remained but a very short time at a maximum. He considered the colour "greenish, almost blue" by comparison with Lalande 42,304, not far distant.

From *Nature*, 15, December 14, 146; 1876

review article

Significant journals of science

Eugene Garfield*

In 1974 the Science Citation Index (SCI) covered about 401,000 articles and communications in 2,443 scientific and technical journals. They cited about 3.2 million different publications an average of 1.8 times each. In this article some results of an analysis of more than 5 million citations in the references of journal articles indexed for the SCI in 1974 are presented and an attempt is made to interpret of those results in the light of an earlier study of 1969 citations.

THE basic information recorded in the *SCI* for citing and cited papers is a "condensed citation." It gives first author, year, journal, volume, and page. The citing-cited pairs can be sorted and subsorted in various ways, as one's interests dictate. Sorting by cited author produces the *Citation Index* section of the *SCI*. Sorting by citing and cited journals produces the two major sections of the *Journal Citation Reports (JCR)*.

ISI's *Journal Citation Reports* is an index of journal-journal links based on a grouping and summation of condensed citations using journal rather than author as the primary sorting key. A preliminary *JCR*, based on an analysis of 1969 references¹, appeared in 1972². This year the *JCR* became a regular section and volume of the *SCI*³. It is the source of the 1974 citation data discussed here.

In this report I have used two indicators of journal significance: total citations and impact. The first is simply the number of times a journal was cited in 1974. Impact, on the other hand, is a measure of the relationship between citations and articles published. For this report, impact was calculated by dividing the number of 1974 citations of 1972 and 1973 articles by the number of articles published in 1972 and 1973. For example, the 817 articles published in 1972 and 1973 in the *Journal of Molecular Biology* were cited 6,129 times in 1974. The impact of the journal is therefore 7.502.

Fig. 1(a) lists the 206 journals most cited in 1974. Fig. 1(b) lists an additional 78 journals whose 1972 and 1973 articles only—rather than articles of any and all years, as in Fig. 1(a)—were highly cited in 1974. (The total of 284 journals in Fig. 1(a) and (b) corresponds to the number of journals listed in Fig. 2(a) and (b), which have impacts greater than 2.) In most cases (63%) these journals began publication in the 1960s and 70s. Older journals like the *Comptes Rendus* rank well in Fig. 1(a), mainly because there is so much that can be cited. Fig. 1(b) is a needed supplement to the list in Fig. 1(a), since the journals have high current citation but lack historical mass to push them up into the top of a list ranked by total citations.

Figures 2(a) and (b) show the 284 journals with impacts greater than 2. Fig. 2(a) lists 206 primary journals. Fig. 2(b) lists 78 review journals; the impact of review journals is generally higher than that of primary journals.

Figure 3 lists journals that rank highest in citation and

impact for three specialities: mathematics, botany, and astronomy/astrophysics. The differences in average impact and citation between the three illustrative categories indicate why comparisons between journals in different specialities may be invidious. For example, it would be foolish to conclude merely on the basis of citation counts that *Astrophysical Journal* is a "better" journal than *Annals of Mathematics*, or to hypothesise without a great deal of study which serves its own field "better."

Variation from field to field is determined by the interplay of several factors. Perhaps the most important is the average number of references per paper in the field⁴. In general, mathematicians cite less than half as many papers as do biochemists. Engineers on the other hand cite books as heavily as journals, as do social scientists. Furthermore, calculation of impact based on 1972 and 1973 publications is bound to affect the impact of journals in a field like mathematics, where citation of older literature is far more common than in others. Thus, the impact of mathematics journals would be higher if calculated on the basis of 1970 and 1971 publications.

It seems necessary to point out the obvious, as I have done in preparing Fig. 3, because one short-sighted criticism of the *JCR* has been that its listings and rankings are undiscriminating. One can get from the *JCR* information on journals within disciplines for intradisciplinary comparison. None of the mathematics journals listed in Fig. 3 was cited enough to appear in Fig. 1(a), but the citation counts and impact factors show plainly that the two leading mathematics journals are *Transactions of the American Mathematical Society* (on the basis of total citations) and *Acta Mathematica* (on the basis of impact). In both citation and impact the average mathematics journal ranks lower than the average astronomy or botany journal.

If one wishes to add to a general-science collection the two or three leading journals of mathematics, botany, or astronomy/astrophysics, one must examine longer lists and select from them the top journals in each speciality, as I have done in preparing Fig. 3.

The remarkable stability of the significant journals of science is attested by their continued high citation and impact. Of the 206 journals most cited in 1969, 169 remain among the top 206 in 1974. One may regard the changes as the result of healthy competition. The 37 journals that dropped from the 206 most cited between 1969 and 1974 rank between 224 and 426 in the complete listing that appears in the *JCR*⁵.

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Fig. 1a. Journals most highly cited in 1974. Journals are listed in descending numerical order of total citations in the references of 1974 issues of journals processed for the *Science Citation Index*. A: rank in terms of total 1974 citations. B: rank in terms of total 1969 citations. C: total 1974 citations. D: 1974 impact. E: total 1974 citations of 1972 and 1973 articles. F: rank in terms of 1974 citations of 1972 and 1973 articles. An asterisk before a journal title indicates that counts for sections, retitled continuations, translated versions, and so on, have been combined with those for the original; the number in parentheses after the journal title indicates the number of such sections, and so on, that went into the combination, including the original. *b.* Journals whose 1972 and 1973 articles were highly cited in 1974. Journals are listed in descending numerical order of total 1974 citations of their 1972 and 1973 articles. Journals ranking higher in this respect will be found among the journals listed in Fig. 1a. See the legend of Fig. 1a for significance of the column markers. An asterisk before a journal title indicates that counts for sections, retitled continuations, translated versions, etc., have been combined with those for the original; the number in parentheses after the journal title indicates the number of such sections, etc., that went into the combination, including the original. The date of a journal's inauguration follows its title.

a													
A	B	C	D	E	G	H							
1	1	98995	J. Am. chem. Soc.	4.383	17088	3	58	87	10206	Analytical Biochem.	2.379	2184	64
2	2	91645	*Physical Rev. (5)	2.670	19174	1	59	46	9824	*Dokl. Akad. Nauk SSSR (7)	0.339	1681	81
3	3	81353	J. biol. Chem.	5.843	13685	6	60	62	9779	Am. J. Med.	4.411	1535	90
4	5	75206	*Nature (3)	4.006	18924	2	61	76	9678	J. natn. Cancer Inst.	3.289	2858	52
5	4	66272	*J. chem. Soc. (9)	1.870	12513	7	62	95	9497	Cancer	2.361	2056	66
6	6	62041	J. chem. Physics	2.918	10462	9	63	59	9142	Can. J. Chem.	1.396	1793	73
7	8	51491	Biochim. biophys. Acta	3.120	14129	5	64	707	9094	FEBS Letters	3.049	4815	25
8	7	47505	Science	5.412	11781	8	65	74	9082	Circulation Res.	4.922	1698	79
9	9	46917	Proc. natn. Acad. Sci. USA	8.989	15317	4	66	108	9026	*Physica Status Sol. (3)	1.476	3201	44
10	11	37047	Lancet	6.677	10383	10	67	64	8903	Tetrahedron	1.576	1913	69
11	10	31563	Biochem. J.	3.627	4885	23	68	77	8890	Am. J. Obstet. Gynec.	2.100	2236	62
12	12	29275	Physical Rev. Letters	5.059	10108	11	69	78	8835	Plant Physiol.	2.580	1935	68
13	32	27080	Biochemistry	4.711	7325	17	70	54	8803	*Acta chem. scand. (3)	1.042	1192	124
14	25	26726	New Engl. J. Med.	8.364	7385	15	71	63	8798	J. Lab. clin. Med.	2.802	1132	131
15	22	24768	J. clin. Invest.	6.992	5377	21	72	113	8693	Gastroenterology	5.394	2260	61
16	18	24209	J. molec. Biol.	7.502	6129	18	73	107	8625	Appl. Physics Letters	3.220	3246	43
17	41	23220	Biochem. biophys. Res. Comm.	3.744	8110	12	74	70	8619	J. appl. Physiol.	1.780	1184	125
18	19	22520	J. Physiol. Lond.	4.495	3160	46	75	481	8478	Applied Physics Letters	2.403	4205	31
19	33	22460	*Nuclear Physics (3)	2.514	7356	16	76	141	8241	J. organomet. Chem.	2.392	3891	35
20	21	22245	*J. Cell Biol. (2)	6.770	3683	38	77	56	8183	Bull. Soc. chim. France	1.001	1492	96
21	29	22201	Astrophys. J.	4.063	7451	14	78	81	7941	Bull. chem. Soc. Japan	0.932	1859	72
22	14	21519	Am. J. Physiol.	2.414	2412	59	79	132	7928	J. Chromatography	2.173	2886	51
23	27	20748	Brit. med. J.	3.556	4829	24	80	71	7922	Acta physiol. scand.	2.204	919	170
24	36	20699	J. expl. Med.	11.874	5557	19	81	72	7914	J. phys. Soc. Japan	1.132	1500	95
25	15	20539	J. org. Chem.	1.495	3526	40	82	61	7860	*Z. Naturforschung (3)	1.070	1503	94
26	16	19277	J. appl. Physics	1.558	3275	42	83	192	7794	J. Neurochem.	3.535	2464	57
27	31	18375	J. Bacteriology	2.727	3809	37	84	106	7656	*Br. J. Pharmacol.	3.516	1751	77
28	30	18190	Analytical Chem.	3.291	4140	32	85	80	7459	Ann. Surgery	2.129	1060	140
29	17	18171	Proc. Soc. expl. Biol. Med.	1.471	2454	58	86	113	7335	*Cell Tissue Res. (2)	1.961	1761	75
30	23	18086	J. phys. Chem.	2.031	2768	54	87	122	7183	J. Pediatrics	2.600	1890	70
31	26	17211	J. Am. med. Ass.	3.068	2982	49	88	84	7120	Blood	4.319	1529	91
32	20	17201	*Proc. R. Soc. (3)	2.350	1114	135	89	60	7117	Helv. chim. Acta	1.649	1034	144
33	13	16782	*C.R. Acad. Sci. (5)	0.529	4247	29	90	68	7063	Philosophical Mag	1.836	876	178
34	35	16509	Tetrahedron Letters	1.777	5004	22	91	147	7007	Biochem. Pharmacol.	2.023	1689	80
35	38	15970	*Archs Biochem. Biophys. (2)	2.952	3050	48	92	100	6951	Pediatrics	2.502	1346	105
36	53	15948	Endocrinology	4.337	4098	33	93	120	6811	Am. J. Cardiol.	3.704	1889	71
37	49	15826	J. Immunology	5.112	4703	26	94	276	6788	J. Virology	4.864	3142	47
38	34	15666	*Physics Letters (2)	2.133	7672	13	95	149	6770	*J. Bone Jt Surg. (3)	1.358	729	234
39	39	15281	J. geophys. Res.	2.536	3854	36	96	73	6662	Z. Physik	1.340	864	182
40	24	14706	*Chem. Ber. (2)	1.506	1353	104	97	112	6600	Experientia	0.883	1647	83
41	37	14668	Ann. N. Y. Acad. Sci.	1.181	1291	113	98	88	6539	J. gen. Physiol.	4.308	741	229
42	52	14461	Circulation	6.834	4025	34	99	51	6362	*Fizika tverd. Tela (2)	0.762	1388	102
43	50	14310	Inorg. Chem.	2.457	3589	39	100	129	6307	Radiology	1.198	1320	107
44	45	13911	*Acta crystallographica (3)	1.361	2394	60	101	66	6177	Annln Chemie (J. Liebig)	1.024	432	379
45	82	13847	*Eur. J. Biochem. (2)	3.857	4595	27	102	89	6066	*Archs internal Med. (2)	2.202	946	163
46	47	13753	J. Pharmacol. expl. Ther.	3.576	2026	65	103	90	5994	Am. Heart J.	1.791	840	188
47	42	13072	Fedn Proc.	0.489	4212	30	104	86	5885	J. opt. Soc. Am.	2.016	905	173
48	58	12544	Cancer Res.	3.391	3164	45	105	94	5849	*J. Physics Chem. Solids (2)	1.394	715	239
49	69	11645	*J. clin. Endocr. Metab. (2)	5.170	3443	41	106	99	5761	J. inorg. nucl. Chem.	0.962	1149	128
50	43	11459	*J. Physics (7)	1.689	5450	20	107	156	5743	J. Endocrinology	2.919	1757	76
51	28	11421	*Zh. eksp. teor. Fiz. (2)	1.565	1607	84	108	217	5683	*J. Pharmaceut. Sci. (3)	1.622	1549	92
52	57	11371	Virology	3.752	2949	50	109	92	5679	J. gen. Microbiol.	2.160	1136	129
53	40	11294	*J. Polym. Sci. (6)	0.964	1565	88	110	115	5675	Surgery	1.559	842	187
54	65	11127	Exp. Cell Res.	3.014	2788	53	111	378	5573	Solid St. Comm.	1.945	2768	55
55	48	10756	*Angew. Chem. (2)	4.140	2666	56	112	170	5557	Clin. chim. Acta	1.669	1587	86
56	67	10231	Ann. internal Med.	4.828	2187	63	113	150	5556	J. Neurophysiology	4.537	676	249
57	355	10227	Brain Res.	3.104	4522	28	114	98	5501	Methods Enzymology	1.765	547	311
							115	136	5491	Archs Surgery	1.462	915	171
							116	101	5486	Surgery Gynec. Obstet.	1.332	750	226
							117	109	5478	J. Electrochem. Soc.	1.053	1098	136
							118	55	5474	*Nuovo Cimento (3)	0.994	999	155
							119	123	5428	J. acoust. Soc. Am.	1.142	830	195
							120	96	5388	Am. J. Pathol.	2.807	856	184
							121	91	5388	J. expl. Psychol.	1.027	750	226
							122	126	5363	*Spectrochim. Acta (3)	1.487	840	188
							123	83	5326	Genetics	2.835	995	157
							124	158	5197	J. Ultrastruct. Res.	2.709	837	190
							125	103	5186	Revs mod. Physics	21.500	731	231
							126	121	5167	J. Histochem. Cytochem.	4.005	757	224
							127	102	5138	Anat. Rec.	2.884	649	265
							128	235	5092	*Zh. obsch. Khim. (2)	0.808	1050	142
							129	192	5063	Immunology	2.816	1118	132
							130	125	5053	J. Nutrition	1.845	740	230
							131	117	5038	Am. J. Roentg. Rad. Ther.	1.008	634	272
							132	166	5033	J. Lipid Res.	3.525	719	238
							133	134	5031	J. Urology	0.721	776	216
							134	194	5000	Life Sciences	2.062	1200	121
							135	177	4909	Acta endocrinologica	2.461	1383	103
							136	267	4861	J. infect. Dis.	3.040	1669	82
							137	75	4847	Phytopathology	1.155	789	210
							138	111	4822	Physics Fluids	1.188	972	159
							139	116	4801	Rev. scient. Instrum.	1.018	1001	153

140	160	4767	J. Biochem. Japan	1.715	1079	138	282	589	2831	Solar Physics 1967	1.929	1059	141
141	184	4707	Nucl. Instrum. Meth.	1.050	1420	100	291	191	2725	NS Archs. Pharmacol. 1972	2.792	1033	145
142	127	4704	Z. anorg. allg. Chem.	1.019	593	286	230	319	3403	Annu. Rev. Biochem. 1932	19.358	1026	147
143	159	4697	J. comp. Neurol.	3.725	771	219	267	190	2946	Archs gen. Psychiatry 1960	2.475	1022	150
144	105	4656	Can. J. Physics	1.038	774	218	237	500	3279	Psychopharmacologia 1959	2.347	1002	152
145	168	4655	Lab. Investigation	2.940	932	166	352	776	2326	*Zh. analyt. Khim. 1946 (2)	1.060	996	156
146	133	4604	Hoppe-Seyler's Z. physiol. Chem.	2.291	1031	146	370	416	2171	IEEE J. Quantum Electronics 1965	3.567	988	158
147	211	4603	Applied Optics	1.832	1539	89	232	327	3373	Biopolymers 1963	2.492	972	159
148	370	4600	Surface Science	3.340	1787	74	459	—	1592	Transplantation Revs 1969	25.579	972	159
149	224	4511	*Comp. Biochem. Physiol. (3)	1.014	1250	116	256	326	3074	Chromosoma 1959	3.875	961	162
150	247	4480	Applied Microbiology	1.292	1196	122	356	—	2265	Metallurg. Trans. AIME 1970	1.054	939	164
151	155	4479	Am. J. clin. Pathol.	1.348	663	255	434	—	1707	*Lettere Nuovo Cimento 1969 (2)	0.755	929	167
152	182	4462	Am. J. Surg.	1.183	731	231	357	627	2258	J. gen. Virology 1967	2.501	928	168
153	220	4453	Molecular Physics	2.334	1258	115	468	—	1553	Optics Communications 1969	1.551	920	169
154	442	4451	*J. comp. Physiol. (2)	2.782	893	175	311	345	2551	*Chest 1970 (2)	1.253	916	172
155	137	4416	Am. J. Dis. Child.	1.495	809	202	323	505	2422	Mutation Res. 1964	2.365	894	174
156	162	4393	*Archs Dermatology (3)	1.784	835	192	289	533	2738	Accts chem. Res 1968	7.403	881	177
157	262	4369	Phytochemistry	1.103	1568	87	300	394	2630	*Agric. biol. Chem. Tokyo 1961 (2)	0.982	867	179
158	110	4356	Acta Metallurgica	1.705	583	291	305	470	2559	Carbohydrate Res. 1965	1.312	867	179
159	93	4353	*J. comp. physiol. Psychol. (2)	1.230	663	256	420	NA	1771	J. Vacuum Sci. Technol. 1964	1.472	867	179
160	140	4348	Cold Spring Harb. Symp.	2.443	623	278	371	539	2159	*J. chromatogr. Sci. 1969 (2)	3.196	847	186
161	139	4347	Ann. Physics	2.128	598	284	338	512	2387	Earth planetary Sci. Letters 1966	1.802	827	196
162	214	4308	Planta	2.589	1261	114	227	268	3423	Br. J. Haematol. 1955	2.711	824	197
163	135	4303	Archs Pathology	1.521	508	332	321	530	2449	Clin. Pharmacol. Therap. 1970	3.423	818	199
164	85	4277	*Proc. IEEE (2)	2.013	781	215	253	277	3114	Obstet. Gynecol. 1953	1.367	816	200
165	147	4253	Pflugers Arch./Eur. J. Physiol.	1.810	856	184	306	317	2557	Steroids 1963	3.189	810	201
166	238	4208	*J. Pharmacy Pharmacol. (2)	3.140	1118	132	548	—	1281	J. magn. Resonance 1969	2.082	808	203
167	443	4180	*Zh. neorg. Khim (2)	0.523	823	198	274	248	2893	Med. J. Australia 1914	0.725	805	204
168	199	4116	J. Anim. Sci.	1.311	1000	154	304	389	2600	Izv. Akad. Nauk SSSR Khim 1936	0.540	802	205
169	153	4104	Chem. Revs	11.154	580	293	220	361	3530	Expl Neurology 1959	1.827	793	207
170	161	4093	J. thorac. cardiovasc. Surg.	1.480	836	191	427	552	1740	J. Crystal Growth 1967	2.503	791	209
171	180	4072	*J. cell. Physiol. (2)	3.737	710	240	286	381	2767	*J. Obst. Gyn. Br. Comm. 1961 (2)	1.922	786	211
172	286	4068	J. Reprod. Fert.	2.357	1414	101	494	627	1453	Icarus 1962	3.489	785	212
173	274	4054	*Transplantation (2)	2.250	1134	130	277	343	2885	J. Catalysis 1962	1.603	784	213
174	558	4049	Clin. expl Immunol.	4.423	1601	85	428	608	1728	*Pediatric Res. 1967 (2)	4.399	783	214
175	176	4040	Coll. Czech. chem. Comm.	0.791	831	194	384	941	2035	Macromolecules 1968	2.276	776	216
176	169	4031	*Am. Rev. resp. Dis. (3)	1.630	937	165	246	207	3155	Anesthesiology 1940	2.024	771	219
177	189	4023	Geochim. cosmochim. Acta	4.056	1160	127	241	293	3186	J. agric. Food Chem. 1953	1.195	771	219
178	271	4005	Analytica chim. acta	2.093	1312	110	257	311	3069	*J. Atmosph. Sci. 1962 (2)	2.051	769	222
179	157	4003	*Deut. med. Wschr. (2)	1.022	1025	149	390	374	1990	*Fiz. Tekh. Poluprovodn. 1967 (2)	0.680	762	223
180	148	3996	Physiol. Revs	13.861	499	334	357	736	2261	*Zh. org. Khim. 1965 (2)	0.643	757	224
181	138	3993	Acta med. scand.	1.124	508	331	361	399	2222	Talanta 1958	1.787	731	231
182	195	3952	Diabetes	3.941	863	183	267	277	2949	*Can. J. Botany 1951 (2)	1.069	729	234
183	97	3932	*Zh. fiz. Khim (2)	0.331	646	266	251	255	3130	Archs Dis. Childhood 1926	1.901	728	236
184	194	3906	Geol. Soc. Am. Bull.	1.674	1026	147	311	320	2547	Br. J. Cancer 1947	3.232	724	237
185	364	3899	Astronomy Astrophys.	2.267	2018	67	233	164	3333	Makromolekul. Chemie 1945	1.088	704	241
186	172	3897	J. Dairy Sci.	0.273	569	300	425	—	1755	Org. Mass Spectrometry 1968	1.088	704	241
187	218	3892	Neurology	2.181	796	206	259	235	3038	Br. Heart J. 1939	1.631	698	243
188	503	3874	*Int. J. Cancer (2)	4.928	1508	93	270	266	2927	*Nouv. Presse Med. 1972 (2)	0.612	696	244
189	367	3869	Clinical Chem.	3.195	1460	97	387	576	2004	Toxicol. appl. Pharmacol. 1959	1.672	689	245
190	171	3864	Am. J. Ophthalmol.	1.389	792	208	349	334	2332	*Archs Microbiology 1974 (2)	1.468	684	246
190	178	3864	Progr. theor. Physics	1.421	1003	151	281	282	2845	Metabolism 1952	2.387	678	247
192	178	3858	Mon. Not. R. astr. Soc.	2.467	1036	143	828	—	717	Kidney International 1972	3.740	677	248
193	165	3857	Archs Ophthalmology	1.293	561	302	326	639	2433	Expl Brain Res. 1965	3.596	676	249
194	154	3852	J. Fluid Mech.	1.254	617	280	228	259	3414	J. mol. Spectroscopy 1957	1.744	675	251
195	146	3827	*Ber. Bunsenges.	1.382	532	319	371	587	2147	Vision Research 1961	1.800	675	251
196	160	3820	J. math. Physics	1.046	632	274	353	297	2321	Planetary Space Sci. 1959	1.645	671	253
197	339	3777	*J. mednl Chem. (2)	1.444	1196	123	293	183	2696	Can. J. Biochem. 1964	1.671	670	254
198	369	3726	Gut	3.336	1081	137	302	472	2621	Molec. Pharmacol. 1965	3.785	670	254
199	130	3710	Am. J. Botany	1.378	357	441	238	228	3259	J. clin. Pathol. 1947	1.550	662	258
200	232	3701	J. Neurosurgery	1.252	636	271	269	308	2931	J. Insect Physiol. 1957	1.505	662	258
201	204	3699	Scand. J. clin. Lab. Invest.	1.917	644	268	263	308	2987	Am. J. clin. Nutrition 1954	1.714	658	260
202	249	3673	*Archs Neurol. (2)	2.217	745	228	226	185	3453	Austral. J. Chem. 1953	1.006	658	260
203	599	3647	*Eur. J. Pharmacol. (2)	2.537	1205	120	234	281	3321	*J. Cell Sci. 1966 (2)	2.973	657	262
204	339	3633	Developmental Biol.	3.384	1242	117	317	NA	2505	J. Fish. Res. Board Can. 1938	1.053	656	263
205	196	3561	Arzneimittel-Forschung	0.876	833	193	294	230	2669	Bacteriol. Revs 1937	16.795	655	264
206	202	3598	*Clin. Sci. mol. Med. (2)	2.474	762	223							

b

284	—	2809	Cellular Immunol. 1970	4.848	1721	78
244	174	3164	Bull. Am. phys. Soc. 1925	0.347	1459	98
376	—	2094	Eur. J. Immunol. 1971	4.852	1441	99
348	—	2337	Infect. Immunity 1970	2.032	1335	106
272	295	2919	*Clin. Res. 1958 (2)	0.198	1316	108
307	—	2556	Transplantation Proc. 1969	2.709	1314	109
487	—	1470	Prostaglandins 1972	5.247	1296	111
281	448	2850	*Molecular gen. Genetics 1967 (2)	2.699	1293	112
242	370	3182	*J. electroanal. Chem. 1967 (2)	1.567	1222	118
239	744	3238	Physiol. Behavior 1966	1.678	1171	126
335	—	2406	Antimicrob. Ag. Chemother. 1972	2.564	1118	132
323	507	2447	J. nucl. Med. 1960	3.040	1061	139

Perhaps the point to be stressed in presenting these data is the bibliographic law of concentration⁶. When the *SCI* was first reviewed in *Nature* more than a decade ago⁷, the scope of its journal coverage was called into question. I believe time has shown beyond doubt that the important literature of science is encompassed by fewer than 1,000 journals. And even fewer account for the truly significant. Of some 45,000 serials of all kinds received by the British Lending Library, two-thirds are rarely, if ever, subject of request. A small core of about 5,000 accounts for almost 80% of all requests⁸.

Fig. 2 a, High-impact journals in 1974 (excluding review journals). Journals are listed in descending numerical order of 1974 impact factor. **b**, High-impact review journals. A: rank in terms of 1974 impact; B: 1974 impact; C: 1969 impact; D: total 1974 citations of 1972 and 1973 articles; E: total number of 1972 and 1973 articles.

<i>a</i>	A	B	C	D	E						
1	11.874	8.307	J. expl Med.	5557	468	74	3.205	—	Clin. Endocrinology	250	78
2	8.989	8.566	Proc. natn. Acad. Sci. USA	15317	1704	75	3.196	1.312	J. Chromatogr. Sci.	847	265
3	8.364	2.359	New Engl. J. Med.	7385	883	76	3.195	0.683	Clin. Chemistry	1460	457
4	7.502	8.811	J. molec. Biol.	6129	817	77	3.189	2.454	Steroids	810	254
5	6.992	3.362	J. clin. Invest.	5377	769	78	3.175	—	J. Neurobiology	200	63
6	6.834	1.214	Circulation	4025	589	79	3.144	1.739	Ann. human Genetics	283	90
7	6.770	3.386	J. Cell Biol	3683	544	80	2.140	1.256	J. Pharmacy Pharmacol.	1118	356
8	6.677	1.485	Lancet	10383	1555	81	3.137	2.593	Am. J. human Genetics	436	139
9	5.843	6.059	J. biol. Chem.	13685	2342	82	3.135	1.981	Am. Naturalist	326	104
10	5.412	2.993	Science	11781	2177	83	3.120	3.102	Biochim. biophys. Acta	14129	4529
11	5.394	1.147	Gastroenterology	2260	419	84	3.104	3.486	Brain Res.	4522	1457
12	5.247	—	Prostaglandins	1296	247	85	3.068	1.050	J. Am. med. Assoc.	2982	972
13	5.170	3.868	J. clin. Endocr. Metab.	3443	666	86	3.049	NA	FEBS Letters	4815	1579
14	5.112	4.121	J. Immunology	4703	920	87	3.048	—	Differentiation	64	21
15	5.059	4.911	Physical Rev. Letters	10108	1998	88	3.040	1.000	J. infect. Dis.	1669	549
16	4.957	—	Scand. J. Immunology	570	115	88	3.040	0.505	J. nuclear Med.	1061	349
17	4.928	2.553	Int. J. Cancer	1508	306	90	3.016	—	Cognitive Psychology	190	63
18	4.922	1.750	Circulation Res.	1698	345	91	3.014	2.241	Expl Cell Res.	2788	925
19	4.864	5.269	J. Virology	3142	646	92	2.973	4.918	J. Cell Science	657	221
20	4.852	—	Eur. J. Immunology	1441	297	93	2.967	3.230	Arch. Biochem. Biophys.	3050	1028
21	4.848	—	Cell. Immunology	1721	355	94	2.940	2.008	Lab. Investigation	932	317
22	4.828	1.679	Ann. internal Med.	2187	453	95	2.920	—	Bioinorganic Chem.	73	25
23	4.711	5.694	Biochemistry	7325	1555	96	2.919	2.021	J. Endocrinology	1757	602
24	4.537	4.435	J. Neurophysiology	676	149	97	2.918	3.128	J. chem. Physics	10462	3585
25	4.495	2.432	J. Physiol. Lond.	3166	703	98	2.916	—	Biol. Reproduction	592	203
26	4.423	3.363	Clin. expl Immunology	1601	362	99	2.884	0.409	Anat. Rec.	649	225
27	4.411	4.516	Am. J. Med.	1535	348	100	2.864	1.337	J. Neuropathol. expl Neurol.	232	81
28	4.399	0.680	Pediatric Res.	783	178	101	2.846	4.057	Q. J. Med.	222	78
29	4.383	5.164	J. Am. chem. Soc.	17088	3899	102	2.835	1.815	Genetics	995	351
30	4.340	NA	Seminars Hematology	204	47	103	2.823	—	J. immunol. Meth.	223	79
31	4.337	2.906	Endocrinology	4098	945	104	2.816	3.859	Immunology	1118	397
32	4.319	2.219	Blood	1529	354	105	2.807	1.814	Am. J. Pathol.	856	305
33	4.380	2.968	J. gen. Physiol.	741	172	106	2.802	1.702	J. Lab. clin. Med.	1132	404
34	4.140	2.925	Angew. Chemie	2666	644	107	2.792	1.266	NS Arch. Pharmacol.	1033	370
35	4.063	4.661	Astrophys. J.	7451	1834	108	2.782	1.638	J. comp. Physiol.	893	321
36	4.060	0.672	Arthritis Rheumatism	613	151	109	2.727	3.341	J. Bacteriology	3809	1397
37	4.056	2.725	Geochim. cosmochim. Acta	1160	286	110	2.711	2.658	Br. J. Hematol	824	304
38	4.006	2.342	Nature	18924	4724	111	2.709	3.012	J. Ultrastruct. Res.	837	309
39	4.005	2.287	J. Histochem. Cytochem.	757	189	111	2.709	—	Transplantation Proc.	1314	144
40	3.967	2.090	Cytogenet. Cell Genetics	357	90	113	2.704	3.596	Physical Rev.	19174	7092
41	3.941	2.039	Diabetes	863	219	114	2.699	2.880	Molecular gen. Genetics	1293	479
42	3.875	2.767	Chromosoma	961	248	115	2.600	—	Intervirology	91	35
43	3.875	3.976	Eur. J. Biochem.	4595	1186	115	2.600	1.374	J. Pediatrics	1890	727
44	3.796	—	Tissue Antigens	429	113	117	2.589	2.944	Planta	1261	487
45	3.785	3.916	Molecular Pharmacol.	670	177	118	2.580	1.573	Plant Physiol	1935	750
46	3.752	4.486	Virology	2949	786	119	2.564	—	Antimicrob. Agents Chemother	1118	436
47	3.744	4.292	Biochem. biophys. Res. Comm.	8110	2166	120	2.545	0.916	Biophysical J.	514	202
48	3.740	—	Kidney International	677	181	120	2.545	—	Eur. J. clin. Invest.	280	110
49	3.737	3.488	J. cell. Physiol.	710	190	120	2.545	—	J. molecular Evolution	112	44
50	3.726	—	Clin. Immunol. Immunopathol.	231	62	123	2.537	3.661	Eur. J. Pharmacol.	1205	475
51	3.725	2.335	J. comp. Neurology	771	207	124	2.536	3.385	J. geophys. Res.	3854	1520
52	3.704	2.170	Am. J. Cardiology	1889	510	125	2.528	—	Radiation Effects	493	195
53	3.627	3.060	Biochem. J.	4885	1347	125	2.528	2.836	Nuclear Physics	7356	2910
54	3.596	4.783	Expl Brain Res.	676	188	127	2.513	—	Thrombosis Res.	392	156
55	3.576	3.568	J. Pharmacol. expl Ther.	2060	576	128	2.512	4.965	J. Petrology	103	41
56	3.567	1.307	IEEE J. Quantum Electronics	988	277	129	2.503	2.277	J. Crystal Growth	791	316
57	3.556	0.677	Br. med. J.	4829	1358	130	2.502	1.495	Pediatrics	1346	538
58	3.535	2.884	J. Neurochemistry	2464	697	131	2.501	2.894	J. gen. Virology	928	371
59	3.525	3.876	J. Lipid Res.	719	204	132	2.492	2.791	Biopolymers	972	390
60	3.516	2.658	Br. J. Pharmacol.	1751	498	133	2.484	3.232	Immunochemistry	611	246
61	3.489	1.697	Icarus	785	225	134	2.481	NA	In Vitro	258	104
62	3.441	3.401	Br. med. Bull.	320	93	135	2.475	1.409	Archs gen. Psychiatry	1022	413
63	3.423	1.657	Clin. Pharmacol. Ther.	818	239	136	2.474	2.732	Clin. Sci. mol. Med.	762	223
64	3.391	2.879	Cancer Res.	3164	933	137	2.467	4.307	Mon. Not. R. astr. Soc.	1036	420
65	3.384	3.729	Developmental Biol.	1242	367	138	2.464	—	Expl Hematology	69	28
66	3.340	2.629	Surface Science	1787	535	139	2.461	1.316	Acta endocrinologica	1383	562
67	3.336	1.174	Gut	1081	324	140	2.457	3.188	Inorg. Chemistry	3589	1461
68	3.291	1.605	Analyt. Chem.	4140	1258	141	2.447	2.873	Neuroendocrinology	438	179
69	3.289	4.009	J. natn. Cancer Inst.	2858	869	142	2.443	5.463	Cold Spring Harbor Symp.	623	255
70	3.266	—	J. Membrane Biol.	578	177	143	2.441	1.685	Neuropharmacology	554	227
71	3.232	1.670	Br. J. Cancer	724	224	144	2.414	3.115	Am. J. Physiology	2412	999
72	3.220	3.545	Applied Physics Letters	3246	1008	145	2.413	—	Hormones Behavior	193	80
73	3.215	NA	J. Allergy clin. Immunol.	463	144	146	2.403	2.477	Chem. Physics Letters	4205	1750
						147	2.392	3.497	J. organomet. Chem.	3891	1627
						148	2.387	2.088	Metabolism	678	284
						149	2.379	3.330	Analyt. Biochem.	2184	918
						150	2.375	0.326	Am. Zoologist	342	144
						151	2.365	2.497	Mutation Res.	894	378
						152	2.361	2.064	Cancer	2056	871
						153	2.357	2.014	J. Reprod. Fert.	1414	600
						154	2.355	NA	J. psychiat. Res.	73	31
						155	2.350	3.085	Proc. R. Soc. Lond.	1114	474

156	2.349	3.662	Psychol. Bull.	444	189	27	6.433	9.600	Adv. Enzymology	193	30
157	2.347	2.380	Psychopharmacologia	1002	427	28	6.357	3.384	Erg. physiol. biol. Chem. exp. Pharm.	89	14
158	2.337		Drug Metab. Disposition	236	101	29	6.133	NA	Adv. organomet. Chem.	92	15
159	2.334	2.173	Molecular Physics	1258	539	30	6.083	18.000	Prog. phys. org. Chem.	73	12
160	2.311	2.561	Faraday Disc. chem. Soc.	208	90	31	6.000	NA	Topics Stereochem.	24	4
161	2.297	1.374	J. Verbal Learning Verbal Behav.	395	172	32	5.733		Annu. Rev. Biophys. Bioenerg	172	30
162	2.291	1.636	Hoppe-Seyler's Z. physiol. Chem.	1031	450	33	5.689		Chem. Soc. Revs	256	45
163	2.286		Organic Mass Spectrometry	704	308	34	5.500	NA	Int. Rev. Cytology	209	38
164	2.279		J. Neurocytology	139	61	35	5.444		Adv. cell. molec. Biol.	49	9
165	2.276	2.529	Macromolecules	776	341	36	5.214		Q. Rev. Biophysics	73	14
166	2.268	2.061	Photochem. Photobiol.	542	239	37	5.045	NA	Adv. Quantum Chem.	111	22
167	2.267	0.987	Astronomy Astrophysics	2018	890	38	5.000	NA	Adv. Colloid Interface Sci.	25	5
167	2.267		J. Steroid Biochem.	390	172	38	5.000	NA	Electroanal. Chem.	15	3
169	2.262	0.842	Invest. Ophthalmology	579	256	38	5.000	3.647	Vitamins Hormones	55	11
170	2.250	3.164	Transplantation	1134	504	41	4.923		Adv. cyclic Nucleotide Res.	256	52
171	2.237	0.869	Gen. comp. Endocrinol.	633	283	42	4.775	6.545	Annu. Rev. Microbiol.	191	40
172	2.234		Cell Tissue Kinetics	239	107	43	4.690	5.176	Biol. Revs Cambridge Phil. Soc.	136	29
173	2.217	1.449	Archs Neurology	745	336	44	4.500	16.285	Solid St. Physics	45	10
174	2.205	1.514	Brain	291	132	45	4.375	NA	Int. Rev. Neurobiol.	35	8
175	2.204	2.479	Acta physiol. scand.	919	417	46	4.339	4.685	Rev. Geophys. Space Physics	269	62
176	2.200	1.769	Archs internal Med.	946	430	47	4.300		Adv. Human Genetics	43	10
177	2.199	NA	Analytical Letters	497	226	48	4.188	5.000	Medicine	268	64
178	2.193	NA	Physics Today	182	83	49	4.176	NA	Adv. microb. Physiol.	71	17
179	2.181	0.868	Neurology	796	365	50	4.156	4.433	Psychol. Rev.	320	77
180	2.173	1.271	J. Chromatography	2886	1328	51	4.000	NA	Adv. Lipid Res.	52	13
181	2.160	2.127	J. gen. Microbiology	1136	526	52	3.783	5.629	Annu. Rev. nucl. Sci.	87	23
182	2.151		J. non-crystalline Solids	628	292	53	3.750	4.695	Coordination Chem. Revs	255	68
183	2.147	2.876	Diabetologia	307	143	53	3.750	NA	Prog. med. Virol.	60	16
184	2.134	2.359	Physics Letters	7672	3595	55	3.500	3.555	Annu. Rev. phys. Chem.	133	38
185	2.129	1.613	Ann. Surgery	1060	496	55	3.500	NA	Prog. med. Genetics	49	14
186	2.128	3.089	Ann. Physics	598	281	57	3.462	NA	Prog. Surf. Membrane Sci.	45	13
187	2.100	1.207	Am. J. Obstet. Gynecol.	2236	1065	58	3.412	7.333	Adv. Virus Res.	58	17
188	2.096	NA	Eur. J. clin. Pharmacol.	262	125	59	3.000	NA	Adv. metab. Disorders	21	7
189	2.093	0.965	Analytica chim. Acta	1312	627	59	3.000	3.818	Botanical Rev.	66	21
190	2.090	2.027	Eur. J. Cancer	466	223	59	3.000	NA	Drug Metab. Revs.	42	14
191	2.083	1.787	Acta mathematica	75	36	59	3.000	NA	Essays Biochem.	27	9
192	2.082		J. magnetic Resonance	808	388	59	3.000	NA	Prog. Materials Sci.	15	5
193	2.073	2.252	Expl Eye Res.	537	259	64	2.923	NA	Catalysis Revs	76	26
194	2.071		Cell Differentiation	145	70	65	2.909	4.500	Prog. cardiovasc. Dis.	160	55
195	2.062	1.839	Life Sciences	1200	582	66	2.900	NA	Int. Rev expl Pathol.	29	10
196	2.056		Contraception	368	179	67	2.844	8.296	Rep. Prog. Physics	128	45
197	2.054	1.643	Int. J. Radiation Biol.	456	222	68	2.746	4.235	Annu. Rev. Medicine	173	63
198	2.051	2.016	J. Atmospheric Sci.	769	375	69	2.456	4.000	Adv. Enzyme Regulation	106	43
199	2.041	1.195	J. Antibiotics Tokyo	445	218	70	2.462	0.176	Q. Rev. Biology	64	26
200	2.032		Infection Immunity	1335	657	71	2.273	5.600	Adv. Carbohydr. Chem. Biochem.	25	11
201	2.031	2.329	J. phys. Chem.	2768	1363	72	2.250	2.888	Harvey Lectures	36	16
202	2.024	2.040	Aesthesiology	771	381	73	2.200	NA	Adv. clin. Chem.	22	10
203	2.023	1.888	Biochem. Pharmacol.	1689	835	74	2.188	NA	Adv. Pharmacol.	35	16
204	2.022	1.855	Theor. chim. Acta	645	319	75	2.086	NA	Annu. Rev. Psychol.	73	35
205	2.016	0.904	J. opt. Soc. Am.	905	449	76	2.079	5.485	Annu. Rev. Entomology	79	38
206	2.013	1.372	Proc. Instn electl electr. Engrs	781	388	77	2.071	NA	Applied Spectrosc. Rev.	29	14
						78	2.047	4.914	Annu. Rev. Phytopathol.	88	43

b

1	25.579		Transplantation Revs	972	38
2	22.643	9.600	Adv. Immunology	317	14
3	21.500	4.317	Revs mod. Physics	318	34
4	19.358	17.584	Annu. Rev. Biochem.	1026	53
5	16.795	20.615	Bacteriol. Revs	655	39
6	15.778	NA	Curr. Topics Microbiol.	142	9
7	13.861	17.333	Physiol. Revs	499	36
8	12.545	13.428	Progr. Allergy	138	11
9	11.613	8.592	Rec. Progr. Hormone Res.	360	31
10	11.154	8.160	Chem. Revs	580	52
11	9.700	8.888	Adv. inorganic Chem. Radiochem.	97	10
12	9.577	22.400	Pharmacol. Revs	498	52
13	9.200	3.259	Adv. chem. Physics	92	10
14	8.379	7.743	Annu. Rev. Astr. Astrophys.	243	29
15	7.875	9.176	Prog. Biophys. molec. Biol.	189	24
16	7.833		Curr. Topics cell. Regulation	94	12
17	7.765	20.200	Prog. nucleic Acid Res.	132	17
18	7.403	17.083	Accts chem. Res.	881	119
19	7.375	3.688	Adv. Physics	177	24
20	7.316	7.047	Annu. Rev. Plant Physiol.	278	38
21	7.143	NA	Curr. Topics dev. Biol.	50	7
22	7.000	NA	Annu. Rev. Pharmacol.	329	47
23	6.963	NA	Adv. Cancer Res.	188	27
24	6.679	NA	Annu. Rev. Genetics	187	28
25	6.636	23.000	Adv. Protein Chem.	73	11
26	6.581	4.216	Annu. Rev. Physiol.	204	31

In using the data presented here, one should be aware that we revised our definition of "source items" used to calculate impacts. In 1969 we included as source items much material (editorials, non-scientific and non-technical correspondence, news notes, and so on) that does not by its very nature invite citation in scientific and technical reports. This policy worked to the disadvantage of some major journals. Our redefinition accounts in part for the changed impact in 1974 of journals like *Nature*, *Science*, *Lancet*, *Journal of the American Medical Association*, and *British Medical Journal*.

What is the significance of journal impact? By demonstrating that only 150 journals have impacts greater than 3, I believe we have established the futility of discussions based on the assumption that the average library must acquire and store thousands of journals. Since the average impact in 1974 was 1.015, any of the journals listed in the figures is likely to be a good candidate for selection.

Fig. 2(b) shows clearly the importance of review journals, confirming our earlier studies. Their extraordinary impact, along with a surge in the number of review-type articles and publications, led to ISI's decision to publish *Index to Scientific Reviews*⁹.

Clearly, a large part of the scientific record is of low impact. Only careful study can show whether this fact

Fig. 3 Significant journals in three scientific specialities. Each list gives journal, (A) total 1974 citations, (B) impact factor, (C) total 1974 citations of 1972 and 1973 articles, (D) number of 1972 and 1973 articles. Journals are listed in alphabetical order. The botany journals include all with more than 600 citations or an impact greater than 1. The astronomy/astrophysics journals include all with more than 400 citations or an impact greater than 0.8. The mathematics journals include all with more than 500 citations or an impact greater than 0.5.

BOTANY

Journal	A	B	C	D
Am. J. Botany	3710	1.378	357	127
Ann. Botany	1674	1.069	232	130
Annu. Rev. Phytopathol.	566	2.047	88	21
Annu. Rev. Plant Physiol.	1760	7.316	278	19
Bot. Review	585	3.000	66	5
Can. J. Botany	2897	1.069	729	343
J. expl Botany	1762	1.506	369	120
J. Phycology	653	1.409	193	74
Mycologia	1143	0.607	176	128
New Phytologist	1405	1.158	300	115
Physiol. Plant Pathol.	206	1.152	114	49
Physiol. Plantarum	2617	1.555	479	196
Physiol. Veget.	322	1.172	116	43
Phytochemistry	4369	1.103	1568	624
Phytopathology	4842	1.155	789	372
Plant Cell Physiol.	1223	1.164	327	115
Plant Dis. Reporter	1489	0.413	307	379
Plant Physiology	8835	2.580	1935	373
Planta	4308	2.589	1261	219
Trans. Br. Mycol. Soc.	947	0.610	186	171
Z. Pflanzenphysiol.	1008	1.340	351	180

ASTRONOMY/ASTROPHYSICS

Journal	A	B	C	D
Ann. Geophysique Paris	588	0.786	110	28
Annu. Rev. Astron. Astrophys.	955	8.379	243	17
Astron. Zh.	738	0.435	171	194
Astronomical J.	2383	1.953	545	182
Astronomy Astrophysics	3899	2.267	2018	497
Astrophys. J.	22201	4.063	7451	1040
Astrophys. Letters	879	1.209	347	
Astrophysics Space Sci.	963	1.048	395	194
Earth planetary Sci. Letters	2387	1.802	827	189
EOS Trans. Am. geophys. Union	625	12.967	389	28
Geochim. cosmochim. Acta	4023	4.056	1160	134
Icarus	1453	3.489	785	150
J. atmosph. Sci.	2630	2.051	769	211
J. atmosph. terrest. Physics	1886	1.322	509	210
J. geophys. Res.	15281	2.536	3854	791
J. Spacecraft Rockets	421	0.334	139	199
Mon. Not. R. astron. Soc.	3858	2.467	1036	249
Planetary Space Sci.	2321	1.645	671	155
Publ. astron. Soc. Japan	360	0.874	83	44
Publ. astron. Soc. Pacific	1191	1.081	308	161
Publ. Dominion astrophys. Observatory	136	1.250	10	2
Q. J. R. astron. Soc.	128	0.923	48	20
Solar Physics	2831	1.929	1059	282
Revs. Geophys. Space Physics	872	4.339	269	40
Sov. Astronomy 'AJ	456	0.295	116	194
Space Sci. Revs	637	1.718	177	34
Z. Astrophysik	597	-		

MATHEMATICS

Journal	A	B	C	D
Acta Math.	675	2.083	75	18
Adv. Math.	137	0.647	44	50
Am. J. Math.	1064	0.474	54	38
Ann. Mathematics	1921	1.226	103	35
Bull. Am. math. Soc.	1281	0.516	221	241
Comm. pure appl. Math.	750	0.598	49	25
C. r. Acad. Sci. A	845	0.210	360	688
Duke math. J.	711	0.391	70	86
Indiana Univ. math. J.	207	0.590	111	94
Inventiones math.	383	0.808	105	67
J. Algebra	834	0.775	248	213
J. differential Equations	375	0.610	111	60
J. math. Anal. Appl.	871	0.393	190	235
J. Math. pures appl.	201	0.879	29	27
Math. Annln	1190	0.381	123	145

Math. Computation	602	0.557	107	109
Math. Z.	1150	0.471	164	152
Michigan math. J.	275	0.482	40	38
Pacific J. Math.	1133	0.279	180	239
Phil. Trans. R. Soc. A	1765	1.016	188	43
Proc. Am. math. Soc.	1725	0.304	433	516
Proc. Cambridge phil. Soc.	1348	0.397	91	103
Proc. London math. Soc.	834	0.533	81	78
Q. appl. Math.	538	0.505	49	43
SIAM J. math. Analysis	107	0.467	56	93
SIAM J. num. Analysis	333	0.662	100	89
Studia math.	506	0.491	106	59
Studies appl. Math.	99	0.615	32	20
Trans. Am. math. Soc.	2622	0.488	371	340

supports or contradicts the idea that science is built on the accumulated results of average effort that prepare the way for breakthroughs¹⁰. In any event, the data seem to me to warrant an examination of the cost-effectiveness of the present publishing system. Journals devote to the mass of rarely cited papers the same resources as to the small part that citation analysis shows to be important. Less than 1% of all papers cited will be cited ten or more times in any annual *SCI*. Although more than 40 million references have been processed for the *SCI* during the past fifteen years, only 116,400 papers have been cited ten times or more in any one year.

One would hope that the availability of *Journal Citation Reports* will have a salutary effect on editorial complacency. A change in a journal's citation rate or impact rate is proper reason for editorial concern, admitting that factors beyond editorial control may be responsible. Thus, a drop in the impact of the leading Soviet journal of physics, *Zhurnal Eksperimentalnoi i Teoreticheskoi Fiziki*, or a rise in the impact of *Teploenergetika* (translated as *Thermal Engineering*) may reflect a shift in interest or emphasis of research worldwide. But a change in citation rate or impact rate may just as likely reflect a change in quality of output.

Journal citation analysis can be quite complex in some cases. The problem of Soviet publications is one such case. Apart from the usual bibliographic problems encountered, one must deal with the fact that most leading Soviet journals appear in two versions, Russian and English. *Fizika i Tekhnika Poluprovodnikov* appears in English as *Soviet Physics Semiconductors*. Clearly that is not a close translation of the title, much less a transliteration. Such bibliographic casualness about titles is bad enough, but there is worse. Most of the retitled translations appear about a year after the originals. This means, if one assumes that the translation is the major stimulant of subsequent citation in Western journals, that the citable life of the Soviet literature is unfairly shortened at the outset by an overlong gestation period. And the outset is important, for if an article is going to be cited, it is most likely to be cited during the first or second year after publication. In the case of Russian journals, citations contributed by translated versions are usually out of phase with those of the rest of the literature. To assure confusion worse confounded, some of the translated versions have volume and page numbers different from the Russian originals. In our tabulations for the *JCR*, we have as far as possible compensated for these annoying vagaries.

As the data show, new journals can achieve high impact quickly. Good examples are *Cellular Immunology* (first published 1970) and *Prostaglandins* (1972). Their total 1974 citation counts were 2,809 and 1,470 respectively; their impacts, 4.848 and 5.247. Among the newer journals the 'European' journals are especially notable in this respect. *FEBS Letters* (began 1968, impact 3.049); *European Journal of Biochemistry* (began 1967, impact 3.874); *European Journal of Immunology* (began 1971, impact 4.852). We must hope that internationalisation of journals will continue. I believe that Latin-American, Asian, and African

journals would do well to consolidate in like manner to produce fewer but larger journals. It is clear that a large journal, even if less than first class, is more difficult to ignore than a smaller journal with equal and perhaps greater impact.

In some cases, however, consolidation is inappropriate and may be detrimental. Take, for example, *Journal of the American Chemical Society (JACS)* and *Journal of the Chemical Society*. The *Journal of the Chemical Society* encompasses nine different subtitled journals. If one were to consolidate comparable journals of the American Chemical Society, their total citation count would be about 183,000, almost double the 98,995 of the *JACS*. The impact of this conglomerate would, however, be only 3.381 (respectable enough) rather than 4.383. Insistence by the Chemical Society upon corporate identity for its journals by means of an identical "main title" with repeatedly retitled sections is the source of bibliographic confusion, as well as of much tedious work in sorting out citation data. It seems to me that most commercial publishers would have refused to scrap a title as well-known as *Transactions of the Faraday Society*. In my opinion, the umbrella of a corporate main title for all a society's journals does little for their individual identities.

I have avoided commentary on the performance of specific

journals, preferring to use the space granted me here for data rather than comment and speculation. And I have published many such analyses, usually on a categorical basis in *Current Contents*. All of them have had the same purpose, and lead to the same general conclusion. Science needs objective criteria for measuring the performance of journals. Citation analysis seems to offer a sound beginning. Considering the paucity of management tools available to the average science librarian—general or specialist—and considering as well the often prejudicial role of individual scientists in journal selection (we all have our favourite journals), I feel that the *JCR* data can provide a more reliable basis for journal selection than any we have had until now.

¹ Garfield, E., *Science*, **178**, 471–479 (1972).

² *Journal Citation Reports (JCR)* 1. *Journal Ranking Package*. 2. *Source Data Package*. 3. *Reference Data Package* (Institute for Scientific Information, Philadelphia, 1973).

³ Garfield, E., *Journal Citation Reports: a Bibliometric Analysis of References Processed for the 1974 Science Citation Index*, Science Citation Index 1975 Annual, **9** (Institute for Scientific Information, Philadelphia, 1976).

⁴ Garfield, E., *Current Contents*, **6**, 5–7 (1976).

⁵ Garfield, E., *Journal Citation Reports*, Section 2, 2–4 (1976).

⁶ Garfield, E., *Current Contents*, **31**, 5–6 (1971).

⁷ Cleverden, C. W., *Nature*, **203**, 446 (1964).

⁸ Line, M. B., and Wood, D. N., *J. Documentation*, **3**, 234–245 (1975).

⁹ *Index to Scientific Reviews 1974 Annual, an International Interdisciplinary Index to the Review Literature of Science, Medicine, Agriculture, Technology, and the Behavioral Sciences*, 2 vols (Institute for Scientific Information, Philadelphia, 1975).

¹⁰ Cole, J., and Cole, S., *Science*, **178**, 368–374 (1971).

articles

A model for ocean-floor metamorphism, seismic layering and magnetism

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Ocean-floor metamorphism, which overprints the pseudo-stratigraphy of ophiolite complexes in southern Chile, is best explained by a combination of hydrothermal and contact metamorphism associated with igneous activity at a spreading centre. Such metamorphism may be more important than igneous processes in producing the observed seismic layering and magnetic properties of the ocean floor.

MODELS of accretionary processes of the oceanic lithosphere integrate marine geophysical data, results of oceanic dredging, and studies of the pseudo-stratigraphy and petrology of ophiolites, recognised as sections of oceanic crust^{1–3}. An important element in these models is the relative extent to which primary igneous processes or secondary metamorphism best explain the geophysical properties of the ocean crust. In this paper we describe the overprint of ocean-floor metamorphism on the pseudo-stratigraphy of ophiolite complexes in southern Chile. The metamorphism in these and other ophiolites, as well as in the ocean floor, is better explained by hydrothermal and contact metamorphism associated with igneous processes at a spreading centre than by burial metamorphism⁴. The effects of this special type of metamorphism, as discussed below, may be more important in producing the geophysical properties of the ocean crust than the igneous processes with which they are associated.

Ocean-floor metamorphism

The ophiolite complexes in southern Chile represent the floor of a Mesozoic marginal basin⁵. Their igneous pseudo-

stratigraphy, like that of many ophiolites elsewhere, consists of pillow lavas underlain by a sheeted dyke complex in turn intruded by chemically layered gabbroic plutons (Fig. 1). The complex sequence of continued intrusion and extrusion which leads to the formation of this pseudo-stratigraphy is described elsewhere^{6,7}.

The ophiolite stratigraphy is overprinted by at least two metamorphic episodes, the second of which is a regional event associated with regional deformation, which only affects the rocks surrounding the ophiolites⁸. The associated low-grade regional metamorphism has not significantly altered the ophiolites and its effects can be easily distinguished.

The earlier phase of metamorphism is not related to regional deformation and its effects are restricted to the ophiolites. From the top of the ophiolites, the intensity of metamorphism increases downwards from zeolite facies through greenschist to amphibolite facies within a short vertical sequence of 2–3 km (Fig. 1). Completely fresh pillow lavas at the top of the sequence have not been encountered. The maximum metamorphic grade is attained within the gabbro unit where the metagabbroic rocks can be classified as amphibolites. Below this zone the metamorphic effect dies off rapidly, leaving fresh gabbros. This boundary between fresh gabbro and amphibolite is extremely irregular, deeper penetration of the metamorphic effect being concentrated along fractures, veins, shear zones and dykes. Similar effects have been observed associated with zones of deformation in some of the Italian Apennine ophiolites⁹.

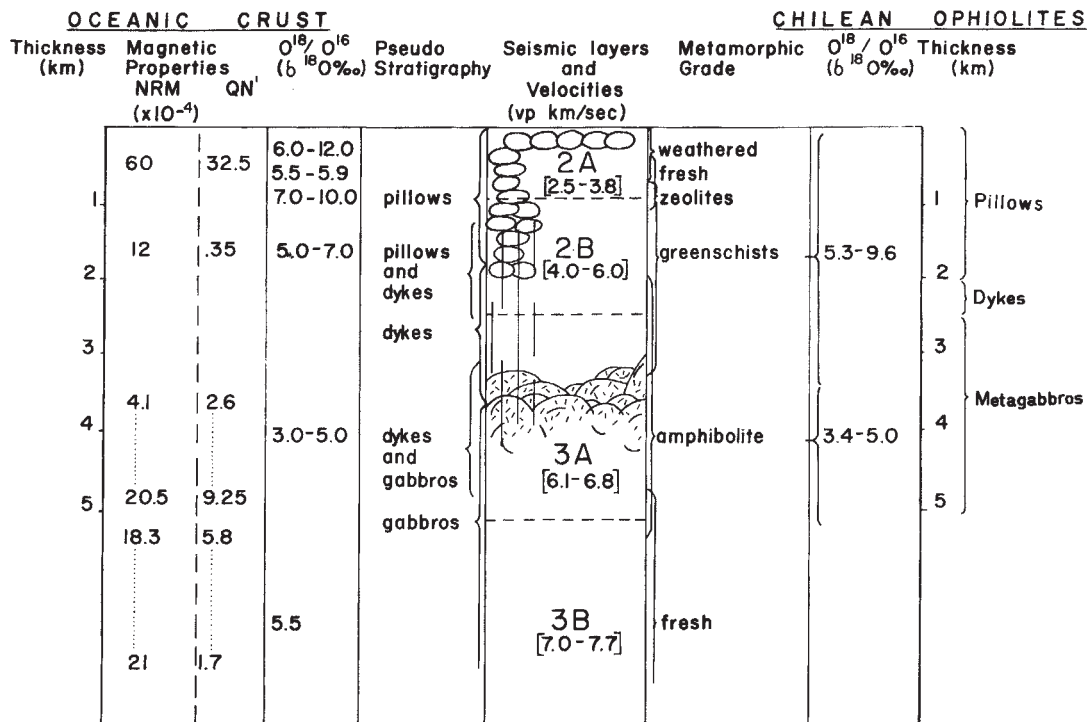


Fig. 1 A schematic column for Chilean ophiolites and a model of oceanic crust. The figure was constructed with preliminary oxygen isotope data for the Chilean ophiolites provided by J. Lawrence, and published seismic²⁹, magnetic³¹, and oxygen isotope²⁰ data which have been correlated with metamorphic zones in the model of oceanic crust.

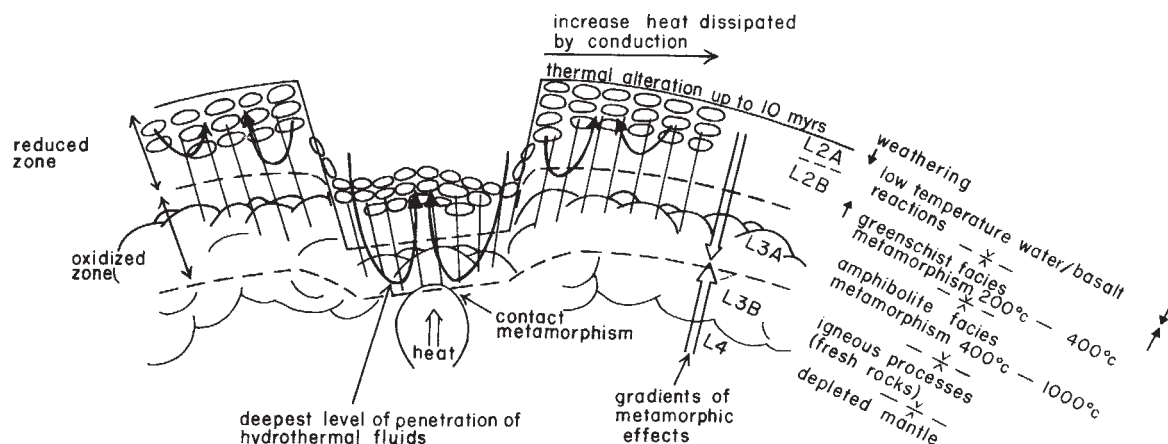
In detail, our petrographic data indicate that at the onset of the lower boundary of the amphibolite-facies metamorphism, olivine in olivine-pyroxene gabbros breaks down to skeletal magnetite and minor serpentine. Pyroxene develops thin rims of hornblende and plagioclase remains essentially unaltered. Higher in the section, pyroxene becomes uraltised and forms iron-rich hornblende. Skeletal networks of secondary magnetite form along fractures and cleavage planes of pyroxenes and amphiboles. Successively upwards, calcic plagioclase is replaced by clino-zoisite, epidote, chlorite, and reacts with silica to form myrmekites. Throughout these states of alteration, the metagabbros retain their original coarse ophitic igneous textures. The overprinted fine-grained metamorphic textures include dendrites and symplectites of epidote, chlorite, and, amphibole; myrmekites and granophyres of low Ca-plagioclase and quartz; and widmanstätten-like textures displayed by amphiboles and opaques. Grain boundaries are often ragged

and feathery. Such textures do not conform with normal textures associated with regional (dynamothermal) amphibolite-facies metamorphism.

Above this zone, metamorphic grade decreases to the greenschist facies. Textures conform with those observed in rocks from other ophiolite terrains and from oceanic dredge hauls. Original igneous textures are not always preserved, and mineralogically the metabasalts and meta-dolerites can be termed spilites⁹⁻¹².

We interpret the observed textures to be the result of metamorphism-metasomatism during which hot aqueous solutions incongruently dissolve and reprecipitate various elements. The breakdown of olivine and the high solubility of silica relative to iron at high temperatures results in the differential removal of Si with respect to Fe. Fe remains partially *in situ* in the form of magnetite, indicating an oxidising environment. The migration of fluids containing both dissolved Si and Fe, upwards along the decreasing

Fig. 2 Model of hydrothermal-contact metamorphism at a spreading centre, as outlined in the text. The figure shows the deep penetration of hydrothermal cells close to the ridge axis and their diminution away from the main zone of igneous activity and tectonism. The deepest level of penetration of the hydrothermal cells defines the depth to the bottom of layer 3A.



thermal gradient, results in the local precipitation of secondary magnetite as well as the formation of iron-rich hornblende from pyroxene. At successively higher levels, lower temperature decreases the solubility of Si, which instigates the reaction between silica and plagioclase to form myrmekites-dendrites and quartz. At still higher levels (dykes and pillow lavas) under lower prevailing temperatures, local metasomatism causes chemical and mineralogical changes termed spilitisation, which have been described previously⁹⁻¹². At this level sulphide mineralisation is common in ophiolites¹³ and the environment seems to be reducing (Fig. 2).

A deuteric rather than hydrothermal process has been suggested as a possible cause for such a sequence of alterations^{11,14}. Conate fluids would be expected to be enriched at the top of magma chambers; however, the estimated weight percent of juvenile waters in oceanic tholeiites is low¹⁵. Hydrothermal circulation of seawater is a more likely explanation for the large scale (volume) alteration⁹. Preliminary data from the Chilean ophiolites (J. Lawrence, unpublished data) show a decrease in $\delta^{18}\text{O}$ from 9‰ in pillow lavas and pillow breccias, to 3.5‰ in the lowest metagabbros. Less altered underlying gabbros have values of 6.5‰, more representative of fresh oceanic gabbros and basalts (Fig. 1). Such a sequence from ^{18}O -enriched to ^{18}O -depleted rocks of basaltic compositions can be explained by re-equilibration, at successively higher temperatures, with a large reservoir of non-magmatic water.

Simple burial metamorphism is unlikely to be the process responsible for these features. Oceanic heat flow data indicate that the geothermal gradient needed to produce the observed metamorphic gradient would be encountered only in a narrow zone restricted to oceanic ridges^{7,16}. Bonatti *et al.*⁸ drew identical conclusions from their study of metagabbros (amphibolites) dredged from the axial valley of part of the mid-Atlantic ridge, which their calculations showed had never been buried more than 1.5 km. They concluded that such a steep metamorphic gradient could be better explained by hydrothermal and contact metamorphism at the oceanic ridges, a process ultimately related to igneous intrusion beneath and volcanism within the median valley of such ridges. We agree with their conclusions and believe that such a model explains the steep metamorphic gradient in the upper section of our and other ophiolites (that is, the Troodos, Apennine, and Bay of Islands Complexes^{8,17}) as well as sections of the oceanic crust⁸. More significantly it can also explain the rapid termination of the metamorphic effects we observe within the gabbroic layer of the Chilean ophiolite complexes.

In simple terms, the model of Bonatti *et al.*⁸ involves a hydrothermal convective cell driven by the heat from igneous intrusions (Fig. 2). Seawater penetrates the oceanic layer along zones of high porosity and structural weakness, to a depth where it is driven back by heat from the intrusion of gabbro and dykes. Extensive hydrothermal systems within active spreading axes are supported by several lines of evidence. First, heat flow observed at spreading centres is lower than theoretical predictions and is best explained by a major component of convective in addition to conductive heat transfer¹⁸. Second, it has been demonstrated that such hydrothermal convective processes are the fundamental cause of metallogenic deposits forming at ridge crests¹⁹. $^{18}\text{O}/^{16}\text{O}$ studies indicate extensive interaction of dredged metabasalt and metagabbro with ocean water²⁰. Such hydrothermal systems have been confirmed by direct observation in Iceland where the spreading centre emerges²¹.

A number of factors indicate that the residence time of the metamorphosed gabbroic rocks within the zone of hydrothermal circulation is short, implying that deeper parts of this zone are narrowly restricted to the spreading

axis. The large surface areas of the myrmekite-dendrite textures indicate rapid growth under supersaturated conditions^{22,23}. Lack of recrystallisation and annealing textures indicates a rapid decrease in local temperature or fluid phase, or both and the absence of retrograde features substantiates this. In metagabbros dredged from the ocean floor, Muehlenbachs and Clayton²⁰ reported disequilibrium between $^{18}\text{O}/^{16}\text{O}$ ratios of coexisting plagioclase and pyroxene which they attributed to a kinetic effect whereby the pyroxene does not react as rapidly as the plagioclase. Such chemical disequilibrium, like textural disequilibrium, implies that the metagabbro had been removed rapidly from the zone of effective metamorphism. Two effects which may combine to restrict the width of the deep zone of hydrothermal alteration are (1) its dependence on intrusive and tectonic activity, which are restricted to near the ridge axis, and (2) the silicification effected by the hydrothermal circulation which acts to reduce the permeability of the rocks. Hydrothermal circulation may exist within a wide area around a spreading ridge, but it is probably confined to the higher level of the oceanic crust, where it causes continued greenschist facies metamorphism and metasomatism (Fig. 2).

Our preferred model for ocean-floor metamorphism near a spreading ridge based on the above evidence is shown in Fig. 2. Deep penetration of hydrothermal fluids is restricted to a narrow zone near the oceanic ridge, with smaller convection cells concentrated at successive higher levels away from the ridge. The greatest depth of penetration of hydrothermal circulation may depend on tectonic activity or spreading rate. Deeper penetration may occur in slow spreading ridges with well developed median valleys than in fast spreading ridges without median valleys. In Chile, metamorphic effects penetrate more deeply in ophiolites in the narrower part of the original basin which may have spread more slowly²⁴. A more complete picture of regional ocean-floor metamorphism is outlined schematically in Fig. 3. This scheme includes dynamothermal metamorphism along fracture zones which is locally complex and polyphasic, as theoretically predicted by Dewey²⁵ and later demonstrated from dredge hauls by Honnorez and Bonatti²⁶. Fracture zones also contain hydrothermal circulation systems hence hydrothermal metamorphic regimes as indicated by the presence of fresh hydrothermal metallogenic deposits²⁷. Also, burial metamorphism *sensu stricto* may play an important part in those areas where sedimentation rate and sediment thickness are high, such as along Atlantic-type continental margins²⁸ and within some marginal basins along Pacific-type continental margins.

Seismic layering and remanent magnetism

Hydrothermal metamorphism may also affect geophysical properties such as the gross seismic velocities and the magnetic properties of the oceanic crust. Models of the velocity structure and lithological character of oceanic crust have been formulated by correlating velocities measured by seismic refraction methods with compressional wave velocity measurements of rocks from the sea floor and ophiolites^{17,29}. Recent seismic work has refined the oceanic layer (layer 3) into an upper layer (3A, 6.0–6.8 km s⁻¹) and a lower layer (3B, 7.0–7.5 km s⁻¹). We suggest that the transition from metagabbros into fresh gabbros marks the transition from seismic layer 3A to layer 3B (Fig. 1). This is consistent with the compressional velocity data and conforms closely with the models of Christensen and Salisbury¹⁷ and a slightly altered model of Peterson *et al.*²⁹.

In our model, this transition marks the depth to which the hydrothermal cells along the spreading ridge have penetrated. Christensen and Salisbury¹⁷ propose that an unaltered gabbro layer is added to an original metagabbro layer by intermittent off-ridge intrusion. Another model is that layer

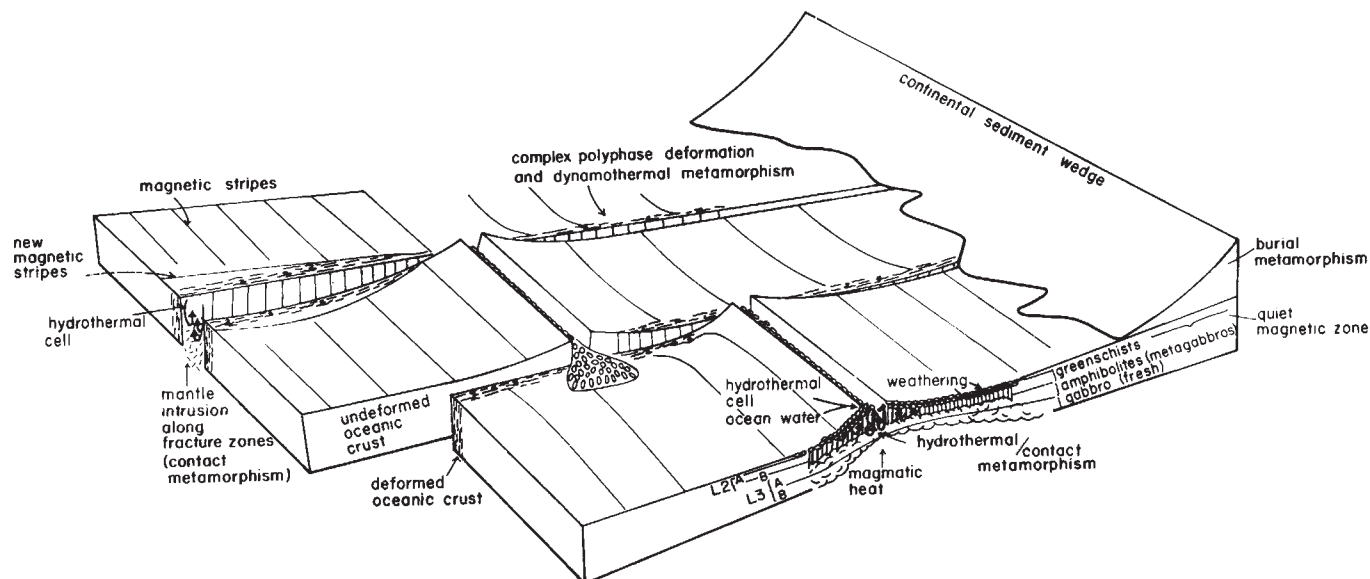


Fig. 3 Schematic illustration of the different elements of ocean-floor metamorphism, each characteristic of a distinct tectonic environment. As new ocean crust is created it undergoes hydrothermal–contact metamorphism at the ridge axis which may be overprinted at later stages by dynamothermal–hydrothermal metamorphism along fracture zones and by burial metamorphism in areas of high sedimentation.

3 is initially entirely amphibolite which becomes progressively dehydrated during further spreading⁴. Our field and textural data on the ophiolite gabbros demonstrate that the opposite sequence occurs. Fresh gabbros are intruded along a central zone and their upper sections are subsequently transformed to metagabbros. This is also supported by studies on other ophiolites such as in Newfoundland (ref. 30 and J. F. Dewey and D. S. Strong, personal communication) and Troodos⁷. Off axis welding of new gabbro and cumulate ultramafic rocks probably does occur and can account for a progressive thickening of layer 3B until a steady-state thickness is reached after about 40 Myr (ref. 17).

From our data in southern Chile, it is clear that both the interface between the gabbro units and the sheeted dykes, and between the sheeted dykes and the pillow lavas is complex and gradational²⁴. The original mineralogy of these overlapping zones tends however, to be homogenised as a result of the metamorphism. Therefore, we also suggest that the boundary between layers 2 and 3 (2B to 3A) marks the transition from amphibolite facies to greenschist facies rocks. Layer 2B includes the part of the sheeted dyke complex (metadolerites) and pillow lavas (metabasalts) which are in the greenschist to prehnite–pumpellyite facies of metamorphism and layer 2A is represented by the fresh to only slightly metamorphosed (zeolite facies) pillow lavas (Fig. 1). Since the metamorphic overprint on the original complex pseudo-stratigraphy of oceanic crust may be dependent on spreading rate, seismic layering may vary from ocean basin to ocean basin.

Models for the preservation of magnetic anomalies are based on measurements of the magnetic susceptibility, the intensity of natural remanent magnetisation (NRM), and the Königsberger ratio (Qn) in rocks drilled and dredged from the ocean floor and sampled from ophiolites^{31–33}. These measurements suggest that fresh quenched pillow basalts with high NRM and Qn are the most likely source of the magnetic anomalies (Fig. 1)³⁴. Nevertheless, even 500 m of fresh pillow lavas are not considered sufficient to produce the measured magnetic anomalies and some contribution must be made by the underlying rocks³⁵.

Our petrographic examination of ocean-floor rocks, for which Fox and Opdyke³¹ have measured the magnetic properties, shows that amphibolitised metagabbros have a higher NRM (18.3×10^{-4} e.m.u. cm^{-3} for partially amphibolitised gabbros V25-D6-39 and 69; 29.4×10^{-4} e.m.u. cm^{-3}

from strongly amphibolitised metagabbros V25-D6-17 and 56) and Qn than either greenschist facies metabasalts (0.12×10^{-4} e.m.u. cm^{-3} for all metabasalts except V25-D5-5 which is only slightly altered to a zeolite facies) and metagabbros (5.7×10^{-4} e.m.u. cm^{-3} for all metagabbros except V25-D5-17 and 56 which are amphibolites) or fresh gabbros (2.1×10^{-4} e.m.u. cm^{-3} for V25-D6-6 and 27) (Fig. 1). Amphibolitised metagabbros could contribute significantly to magnetic anomalies. They contain a high concentration for skeletal growths of secondary magnetite produced by the oxidation and breakdown of olivine and pyroxene and metasomatic redistribution of Fe. Neither completely fresh gabbros nor greenschist facies rocks contain these skeletal networks of secondary opaques.

In our model of the generation and preservation of magnetic anomalies, fresh quenched pillow lavas, basalts, and gabbros all take on the magnetic polarity prevalent during their formation at the ridge axis. Hydrothermal metamorphism alters the igneous rocks, developing amphibolites with abundant secondary opaques in seismic layer 3A, and greenschist to zeolite facies assemblages in the overlying rocks. This occurs in a narrow region restricted to the spreading centre so that the polarity of the entire rock column including fresh pillows is the same. The amphibolites move rapidly out of the deeper hydrothermal metamorphic zone which is narrowly restricted to the spreading axis, effectively quenching their metamorphic textures and NRM polarity. Continued hydrothermal activity and metasomatism is confined to progressively shallower depths away from the ridge and produces greenschists with lowered NRM (Fig. 1) in the overlying rocks. This process does not affect the deeper levels within seismic layer 3A.

With time, the rock column (Fig. 1) moves away from the ridge axis. Ocean-floor weathering and shallow hydrothermal circulation continue to reduce the NRM of the pillow lavas; however, the NRM of the lower metagabbros remains constant. This effect could possibly account for the observed decrease in the amplitude of the linear magnetic anomalies away from the ridge axis for at least 10 Myr followed by a steady amplitude in the anomalies further away from the ridge (ref. 36 and W. C. Pitman III, personal communication).

Hydrothermal circulation within fracture zones could be expected to function in a fashion similar to that at a spreading ridge, although the affected rock assemblages may not be removed as quickly from the higher metamorphic zone,

except by uplift (Fig. 3). Development of secondary opaque minerals, particularly within uplifted serpentinised bodies, would cause these rocks to acquire an overprint of the prevailing magnetic polarity. Cochran³⁷ suggested that the Romanche fracture zone trough is either a zone of reduced magnetisation, or the basement is normally magnetised along the fracture zone for a distance of 500 km. We believe that magnetic overprinting could have caused the latter alternative. Young extension fracture zones, or "leaky" transforms may be expected to give high amplitude anomalies, while prolonged hydrothermal alteration during reversals will alter such anomalies significantly.

High sedimentation rates producing thick sediment blankets will induce a regime of burial metamorphism. Such thick sediment blankets are only associated with particular tectonic environments such as some types of marginal basins, as along the Western Pacific margin, and Atlantic-type continental margins (Fig. 3). The secondary metamorphism that they produce may act to retrograde any magnetic layer in the ocean crust and decrease its NRM. This effect may be one of the reasons responsible for some quiet magnetic zones along continental margins and the absence of well defined linear magnetic anomalies in some marginal basins. Larson *et al.*³⁸ presented similar arguments to account for the absence of magnetic patterns in the northern part of the Gulf of California.

A critical factor in the production of linear magnetic anomalies according to this hypothesis is the regularity of the hydrothermal metamorphism. In a marginal basin, such as the Sea of Japan, extension may not take place along a well defined linear spreading axis, but may proceed within a more diffuse zone with active regions separated by continental blocks. We expect the hydrothermal activity will be as diffuse as the igneous activity with which it is associated. We suggest that this is an additional factor that can account for the irregularity of magnetic anomalies reported within many marginal basins.

Model of metamorphic overprint

The metamorphic overprint on ophiolite complexes in southern Chile, in which metamorphic effects grade rapidly downward from zeolite to amphibolite facies and then disappear abruptly, is better explained by a model of hydrothermal metamorphism closely associated with the zone of igneous intrusion than by the classical concept of regional burial metamorphism. The metamorphic conditions that are reached during this metamorphism are principally a function of temperature and fluid phases. Hence, if the present metamorphic facies concept is to be continued and extended to include ocean floor metamorphism, metamorphic facies must be plotted on three-dimensional diagrams in order to include fluid phase variables, principally H₂O.

The metamorphic overprint may have a more significant influence than the igneous pseudo-stratigraphy in determining some marine geophysical properties. Irregularities in the juxtaposition of the overprint on the pseudo-stratigraphy caused by various rates of spreading, sedimentation affecting hydrothermal circulation, and the linearity or irregularity of spreading activity determined by tectonic environment, all influence the variability of the geophysical properties of the rocks.

The overprint of the metamorphism determines the seismic layering of the ocean floor; seismic layer 2A consists of fresh, weathered, and zeolite facies pillow lavas, basalt and dolerite dykes; seismic layer 3A consists of amphibolite facies dolerite dykes and metagabbros; seismic layer 3B consists of fresh gabbros intruded at the ridge axis but below the greatest depth of penetration of the hydrothermal metamorphism.

The deepest levels of the metamorphism produce an oxidation zone with extensive development of secondary

fine grained opaque minerals which may enable rocks in this zone to preserve a stronger NRM than both underlying fresh gabbros and overlying greenschist, zeolite, and weathered basalts. Marine magnetic anomalies are likely to be produced by an integrated effect of layer 2A and 3A. Layer 3A is not affected by off-axis shallow-level hydrothermal circulation which weathers and spilitises the overlying basalts reducing their NRM. Thus with time this layer may become a more significant contributor to magnetic anomalies.

Irregularities in magnetic anomalies may be related to irregularities in ocean-floor metamorphism caused by (1) high sedimentation such as found along Atlantic-type continental margins, and in some marginal basins, which promotes a new overprint of classical burial metamorphism creating one type of magnetic quiet zones and, (2) diffuseness of the zone of extension such as in incipient rift zones or marginal basins which will result in an irregular pattern of hydrothermal circulation, thus decreasing the likelihood of the generation and/or preservation of linear magnetic anomalies.

Mantle intrusion into extended fracture zones sets off independent regimes of hydrothermal/contact metamorphism and consequently overprints earlier magnetic anomalies in the vicinity of the fracture zones. Magnetic anomalies associated with such fracture zones are expected to be complex, ranging from very high after initial emplacement of uplifted serpentinites to low following extended metamorphism during magnetic reversals.

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Reassessment of roles of oxygen and ultraviolet light in Precambrian evolution

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The rise of atmospheric oxygen occurred long before the sudden appearance of multicellular eukaryotic organisms in the later Precambrian. Oxygen was necessary but not sufficient for the evolution of multicellular eukaryotes: the rise of modern aerobic eukaryotes (fungi, animals and plants) occurred in a fully oxygenic atmosphere only after the evolution in protists of microtubule-utilising processes (mitosis and meiosis).

ALTHOUGH the Earth probably formed about 4.5×10^9 yr ago the oldest rocks found to date are only about 3.5×10^9 yr old¹. Nearly as old as the oldest rocks themselves is the earliest evidence for microbial life; both lithified fossil communities of microorganisms (stromatolites) and silicified remains of bacteria and blue-green algae can be found in sedimentary rocks at least 3×10^9 yr old^{2,3}. Nevertheless the fossil record is extremely sparse during the early period of Earth history. It is not until the beginning of the Phanerozoic era, about 5.8×10^7 yr ago, that a diversity of metazoan and other species suddenly becomes abundant in the fossil record. This abundance of fossils has continued throughout subsequent geological history.

Various explanations have been offered for the sparsity of the Precambrian fossil record and the sudden appearance of abundant fossils at the beginning of the Phanerozoic. The simplicity of two of these suggestions has made them particularly popular. Both are related to the transition from a primitive reducing atmosphere to the modern atmosphere, rich in oxygen. One suggestion⁴⁻⁶, is that atmospheric oxygen became sufficiently abundant to support respiring organisms, including skeleton-forming metazoa, only near the end of the Precambrian. The other suggestion^{4,8} is that Precambrian life was confined to very restricted habitats by the flux of solar ultraviolet radiation that would have bathed the surface of the Earth when the atmosphere lacked sufficient oxygen to produce an ultraviolet screen of ozone. The development of an ozone screen near the end of the Precambrian is presumed to have made possible, for the first time, widespread colonisation of the open ocean accompanied by diversification of life and subsequent formation of abundant fossils.

We argue here that neither of these explanations for the sudden appearance of fossils at the beginning of the Phanerozoic can be accepted. Both geological and biological evidence suggest that the transition from a reducing atmosphere to an oxidising atmosphere occurred long before the end of the Precambrian. In addition, while the metabolic capabilities of prokaryotic organisms (bacteria and blue-

green algae) suggest that they evolved under conditions of changing oxygen tension, the response to oxygen of eukaryotic organisms (which include fungi, animals, complex algae, and plants) is uniform; nearly all of them are aerobic. The implication is that the transition to an oxidising atmosphere preceded the origin of the eukaryotic cells, which in turn must have preceded the origin of metazoa. The accumulation of oxygen in the atmosphere well before the end of the Precambrian would have eliminated solar ultraviolet light as a significant biological factor at the end of the Precambrian. Moreover, the number of methods by which organisms can protect themselves from harmful ultraviolet radiation is sufficiently large to suggest that solar ultraviolet, even when the atmosphere was anaerobic, was more of a tolerable annoyance than a lethal agent that controlled the distribution and diversification of life.

We offer an alternative explanation for the late and apparently sudden appearance of metazoa in lower Cambrian sediments. Our explanation is related to the mechanisms by which fully mature eukaryotic cells (precursors to the metazoa) most probably originated⁹⁻¹¹. There was probably a protracted evolution of modern genetic systems based on mitosis in cells which acquired certain organelles (for example, plastids and mitochondria) by hereditary endosymbiosis^{9,10}. We concur with Cloud¹² that it is the origin of hard parts that underlies the Cambrian explosion of metazoans and we invoke biotic factors intimately associated with the early evolution of eukaryotes to explain the relatively late appearance of metazoa, metaphyta, and fungi.

The rise of oxygen in the atmosphere

It is not likely that abiotic sources of oxygen could have produced an aerobic atmosphere before the origin of oxygen-producing photosynthesis. The only significant abiotic source of atmospheric oxygen¹³ is provided by photolysis of water vapour in the upper atmosphere followed by escape of hydrogen to space. Berkner and Marshall⁷ and Brinkmann¹⁴ made estimates of the level of oxygen in the prebiological atmosphere that could have been maintained by this source. These estimates are almost certainly too high. Both studies erred by assuming that every photolysis is followed by escape. Today, only a small fraction (about 10%) of the hydrogen atoms produced on Earth by photolysis escape. Most of them recombine to form water, leaving no free oxygen. This is true not only in the oxidising atmosphere of the Earth, but also in the less aerobic atmospheres of Mars and Venus¹⁵.

The processes that control the rate of escape of hydrogen from planetary atmospheres have been described by

Hunten¹⁵. Under present conditions, as well as under conditions that are likely to have existed in the primitive terrestrial atmosphere, escape is controlled not by the rate of photochemical production of hydrogen but by the rate at which hydrogen and its compounds are transported upwards from the lower atmosphere to the exosphere. This rate is proportional to the abundance of hydrogen compounds (principally water vapour) in the stratosphere. The abundance of water in the stratosphere is controlled by condensation at the tropopause¹⁶. The abiotic source of oxygen therefore depends on the temperature of the tropopause and not on the rate of photolysis of water vapour. Unless the primitive tropopause was warmer than the present one the abiotic source of oxygen in the primitive atmosphere would have been no larger than it is today. The sources estimated by Berkner and Marshall¹ and by Brinkmann¹⁴ were considerably larger.

In estimating the oxygen budget of the primitive atmosphere these authors also erred by neglecting oxygen consumption in reactions with reduced volcanic gases, principally hydrogen. Today the volcanic hydrogen source seems to be comparable to the rate of escape of hydrogen from atmosphere to space. It is likely to have been larger in the early Precambrian because accretional and radioactive heating should have caused higher temperatures within the Earth. If the volcanic source of hydrogen was larger than the abiotic source of oxygen, then atmospheric oxygen would have been held at very low partial pressures by photochemical reaction with an excess of atmospheric hydrogen. This was presumably the situation when life originated.

The origin of cyanobacterial photosynthesis between 2 and 3×10^9 yr ago introduced a new source of atmospheric oxygen. (Cyanobacteria are blue-green algae¹⁷; from the point of view of photosynthetic oxygen elimination cyanobacterial photosynthesis is the same as green-plant photosynthesis.) This source presumably increased with time. As the interior of the Earth cooled down, moreover, the volcanic hydrogen source may well have decreased. Eventually there came a time when the rate of release of oxygen to the atmosphere exceeded the rate of release of hydrogen. When this threshold was reached the earlier excess of hydrogen would have been replaced by an excess of oxygen. The hydrogen partial pressure would have been driven to low values by photochemical reaction with oxygen. The transition from a reducing atmosphere to an oxidising one could have been quite rapid on the geological time scale.

The time of this transition has been dated at $2.0 \pm 0.2 \times 10^9$ yr ago by Cloud¹⁸. This is the time when red beds replaced banded iron formations in the sedimentary record. Deposition of widespread banded iron formations probably required an anaerobic atmosphere¹⁸; deposition of red beds required an aerobic one¹⁹. How much oxygen is required for the deposition of red beds does not seem to be known. There is, however, biological evidence that the atmosphere contained enough oxygen to support aerobic metabolism well before the origin of metazoa. In fact, Dimroth and Kimberley²⁰ have seriously challenged Cloud's conclusions and have argued that it is even possible that fully oxygenic atmospheric conditions prevailed as far back as the Archean (more than $2,500 \times 10^6$ yr ago).

Genetic variation in related taxa of organisms (for example, species, genera, and families) that can be understood in terms of significant environmental variables can often give insight into the nature of the selection pressures incumbent on the population at the time speciation occurred. Thus, the origin of asymmetry in the flowering parts of angiosperms can be understood in terms of their co-evolution with pollinating mammals, birds and insects; the development of hydrodynamic form in several mammalian lines (dolphins, seals, sea lions, manatees, and whales) can be comprehended as an evolutionary response to increasing periods of the life cycle in aqueous habitats.

The analogous variable response of prokaryotic microorganisms to ambient oxygen is consistent with the view that, as a group, they evolved their major metabolic patterns in response to varying and rising oxygen tensions. Prokaryotes include obligate anaerobes, facultative anaerobes, microaerophils, oxygen-indifferent organisms, and obligate aerobes. Closely related microbes often differ markedly in their response to oxygen, suggesting that evolution of oxygen-handling mechanisms evolved convergently in several prokaryote groups.

By contrast, eukaryotes are extremely uniform in their response to oxygen: they are aerobes. Obligate anaerobes are extremely rare among eukaryotes; there are no metazoan animals or embryophyte green plants that complete their entire life cycles in the total absence of oxygen. No oxygen-indifferent forms have been reported. Among the fungi, a very few are anaerobic and all of these are facultatively aerobic. All eukaryotic anaerobes seem to be derived secondarily from aerobic ancestors. The yeasts, for example, survive and grow fermentatively by reversibly dedifferentiating their mitochondria. Even in anaerobic growth conditions they require metabolites made by aerobic, oxygen-utilising pathways (for example, polyunsaturated fatty acids and sterols). Among the protists only a very few obligate anaerobes or microaerophils are known; some psammophilic ciliates and a number of polymastigote flagellates which, judging from their aerobic, mitochondria-containing, close relatives, seem to have lost their oxygen-utilising mitochondria. Only those that have replaced the lost mitochondria with symbiotic bacteria are able, it seems, to produce the nearly ubiquitous intracellular membranous differentiation known as Golgi apparatus²¹. Furthermore, all of these flagellates are symbiotic forms, primarily in the anaerobic hind-guts of termites. It is surmised that oxygen-requiring metabolites are provided to the symbionts by their hosts or in the diets of the hosts.

The process of mitosis itself requires molecular oxygen²² as does the formation of steroids, polyunsaturated fatty acids that comprise eukaryotic membrane systems, the amino acids tyrosine (at least in animals) and hydroxyproline, as well as hydroxyproline-rich proteins such as collagen and other typical eukaryotic metabolites. Mitochondrial metabolism also involves many oxygen-requiring steps (for example, the squalene oxidase-catalysed production of lanosterol, tyrosine formation, and so forth). It therefore seems likely that full aerobiosis was characteristic of even primitive eukaryotes.

While oxygen was necessary for eukaryotic origin and evolution, there is no evidence that the mere appearance of atmospheric oxygen was sufficient. Complete and obligate aerobiosis is fully documented in many species of prokaryotes²³. As oxygen is depleted both in laboratory and natural communities of microorganisms (on, say, a depth gradient in soil) a distinct correlation can be made of the distribution of the microbial species along that oxygen gradient. Oxygen gradient distribution is less prevalent among the more abundant eukaryotes: they tend to be associated only with fully aerobic or even oxygen supersaturated environments. An easy method to select for prokaryotes and against eukaryotes is simply to fill a container up to overflow with nutrient solutions and place a lid on it. The most reasonable explanation for these phenomena is that oxygen was and still is a selective agent among prokaryotes but that close to modern quantities of oxygen are now and always have been required for eukaryotes. This is consistent with the idea of evolution of major fermentative and respiratory pathways in prokaryotes before and during the transition to the oxidising atmosphere²⁴ and the origin of eukaryotes at a later time.

The origin of the eukaryotic cell after the transition to an oxidising atmosphere, but several hundred million years

at least before the end of the Precambrian^{25,26}, supports the geological evidence that the transition occurred well before the end of the Precambrian. The sudden appearance of abundant fossils at the end of the Precambrian was therefore not associated with the onset of an aerobic atmosphere. The biological evidence, moreover, directly contradicts the idea that the sudden appearance was a result of rising levels of atmospheric oxygen. Prokaryote metabolism appears to have evolved during a period of rising oxygen; the origin of eukaryotic cells, which must have preceded the origin of metazoa composed of these cells, appears to have occurred in an aerobic environment.

Evolutionary effects of ultraviolet light

Biologically harmful solar ultraviolet radiation is absorbed in the terrestrial atmosphere by ozone, produced photochemically from atmospheric oxygen. This ozone screen must have developed gradually as oxygen accumulated in the atmosphere, leading to a changing radiation level in the environment of primitive organisms.

The variation of ozone abundance with the oxygen partial pressure is a problem that would merit study with one of the elaborate photochemical models that have been developed for the investigation of man's impact on the stratosphere²⁷⁻²⁹. Such a study has not yet been conducted, however.

Berker and Marshall⁴ in their investigation of surface ultraviolet during the rise of atmospheric oxygen, made no attempt to solve the problem. They simply assumed a decrease of ozone with oxygen. Ratner and Walker³⁰ used a very simple photochemical model (oxygen reactions only and no transport). They found that the first effect of a decrease in oxygen below present levels is an increase in ozone. The increase occurs because ozone is formed by a three-body reaction which becomes more rapid as the ozone layer moves to lower altitudes. The result is that substantial densities of ozone can exist at quite low oxygen partial pressures. Ratner and Walker found that the atmosphere would contain enough ozone to shield the ground from solar ultraviolet radiation at oxygen partial pressures only 10^{-3} times their modern values. This conclusion might be modified by a study that considered the effects of nitrogen oxides, chlorine, and methane on the ozone photochemistry, but it is impossible to guess whether these complications would increase or decrease the ozone abundance. At present, the best we can do is accept the result of Ratner and Walker³⁰ and conclude that the ozone screen was established before the abundance of atmospheric oxygen reached 1% of its present level. It is clear from the fossil record^{6,31} that this level was reached before 7×10^8 yr ago. Ultraviolet light therefore may well have disappeared from the surface of the Earth long before the end of the Precambrian. In addition, the biological evidence that eukaryotic cells evolved in an aerobic world indicates that ultraviolet light had disappeared from the surface of the Earth even before eukaryotes evolved. We may infer that biologically harmful solar ultraviolet radiation was probably not a factor either in the early evolution of eukaryotic organisms or in the sudden appearance of abundant metazoa at the beginning of the Phanerozoic era.

It is possible, however, that ultraviolet affected the evolution of prokaryotic organisms at even earlier times, before the transition to an oxidising atmosphere. Sagan³² has examined the flux of solar ultraviolet radiation at the ground in a primitive atmosphere devoid of ozone. An unprotected microorganism would be killed in a matter of seconds if exposed to full sunlight. Some scattering and attenuation of radiation by Rayleigh scattering, dust, and water droplets is to be expected³³ but not enough to render the surface habitable. Sagan has shown that an ultraviolet screen could not have been provided by methane, ammonia,

water vapour, carbon dioxide, nitrogen, hydrogen, or hydrogen sulphide. None of these gases absorbs sufficiently strongly at wavelengths near 2,400 Å. Gaseous organic molecules have also been considered by Sagan. They are not likely to have been sufficiently abundant. Sulphur dioxide does absorb strongly throughout the near ultraviolet^{34,35} but its presence is doubtful in the Archean anoxic atmosphere.

The possible screening of organisms by liquid water has been considered by Berkner and Marshall and by Sagan. Pure water absorbs weakly in the ultraviolet; a water thickness of at least a metre would have been needed to protect unshielded organisms in an ozone-free atmosphere, rich in hydrogen sulphide. More than 10 m of water would have been necessary if the atmosphere lacked hydrogen sulphide. On the basis of this work we conclude that inorganic ultraviolet screens were ineffective in the anoxic atmosphere. It is possible that prokaryotic microorganisms, including those that required visible light, may have lived at a safe depth below the surface of the sea, but it is more likely that they used a number of biological mechanisms to protect themselves from ultraviolet damage.

Prokaryotic microorganisms are able to protect themselves against potentially lethal ultraviolet radiation by a variety of means, ranging from avoidance of radiation to enzyme-mediated repair of radiation damage. Negative phototaxis (swimming away from the light source) is one of these mechanisms. It is a behaviour that is known in heterotrophs and non-obligate photoautotrophs as well as microbes as primitive as clostridia, desulphovibrios, and purple non-sulphur photosynthetic bacteria.

Blue-green algae and certain bacteria tend to grow in mats. As suggested by Monty³⁶ it is likely that the matting habit evolved, at least in photoautotrophs, as a protective mechanism against solar ultraviolet light. For example, we have recently observed that large inoculum size (which is equivalent to the matting habit) can protect from death underlying cells in clumps of ultraviolet irradiated blue-green algae. These studies involved *Lyngbya*, very commonly a surface component of algal mat communities. Even after 3 d of continuous ultraviolet irradiation (2×10^5 erg mm⁻², $\lambda_{\text{max}} = 254$ nm), cells on the interior of a mat were entirely viable in conditions in which controls on the surface were killed after minutes^{37,38}. The most common Precambrian fossils, lithified remains of algal mat communities (stromatolites), suggest that the matting habit has been in continuous existence since more than 3×10^9 yr ago³⁶. The surface components of these layers of microbial communities must have afforded excellent protection to underlying layers against potentially lethal solar ultraviolet radiation.

A third method of protection against ultraviolet radiation was discovered in the same experiments. Solutions of simple nitrogenous salts (sodium nitrate and sodium nitrite) absorb strongly in the near ultraviolet. The addition of these salts to the medium in which the algae were immersed was found to protect small amounts of surface filamentous algae from death by irradiation^{37,38}. Survival has been extended greatly by the matting habit or the presence of these nitrogenous salts even in *Lyngbya* directly exposed to the full unfiltered ultraviolet spectrum from a 300-W deuterium lamp at a distance of only 13 ± 0.5 cm (C. Walters, unpublished observations). These protective effects were measured in conditions that prevented photoreactivation. Yet it is well known that ultraviolet damage to nucleic acids calls into play enzymatic repair systems mediated by visible light (photoreactivation). Light-insensitive (dark) repair systems are also known. Such ultraviolet repair systems are apparently universally distributed among bacteria, providing still another mechanism that would have permitted primitive prokaryotic organisms to flourish even in the absence of an atmospheric screen against solar ultraviolet radiation.

The continued presence in many organisms of enzymatic systems for the repair of ultraviolet damage in the absence of any danger of ultraviolet damage has been attributed to the association between ultraviolet sensitivity and lesions in recombination; there is at least one component in the enzyme systems of *E. coli* that controls DNA recombination (breakage and reunion of parent DNA molecules in the formation of new daughter recombinant molecules) and that is also involved in repair after ultraviolet irradiation³⁹. Thus, natural selection for the retention of sexual recombination and DNA repair systems has led to a co-selection for a Precambrian legacy of protection against solar ultraviolet radiation. Bacterial and blue-green algal mutants with high resistance to ultraviolet light have also been reported⁴⁰.

Environmental, behavioural, physiological, and genetic mechanisms for resistance to potentially dangerous ultraviolet light have therefore been established. It is possible that only a fraction of the protection mechanisms have been discovered, but it is clear that a wide range of responses exist even in extant populations in which the threat of dangerous natural ultraviolet irradiation no longer exists. We conclude that potentially lethal solar ultraviolet radiation was a source of selection pressure that led to the stabilisation and retention of several protection mechanisms during and immediately after the origins of prokaryotic cells that it had little effect on the later origin and evolution of eukaryotic cells and higher organisms.

Late appearance of multicellular eukaryotes

Mitosis may be considered to be a process that is a precursor to the sexual and developmental systems of multicellular organisms. Such sexual and developmental processes are based on meiotic-fertilisation life cycles, and meiosis is, in essence, a highly specific and directed variation of mitosis. It is probable, therefore, that the late appearance of metazoan, metaphytan and fungal organisms is, at least in part, a result of the complexity of the mitotic-meiotic system, and the length of time required for it to evolve.

Recent studies on the variations in mitosis and meiosis suggest that these processes evolved in eukaryotic microorganisms, primarily heterotrophs⁴¹. The variations in the mitotic systems of amoebae, dinoflagellates, flagellate algae, radiolarians, ciliates, and amoeboflagellates suggest that stabilisation of the mitotic-meiotic system was a prerequisite for the impressive morphological diversification of metazoa and metaphyta. This stabilisation most probably involved many complicated steps as determined by significant variations in mitosis and sexual life cycles within groups of closely related eukaryotic microorganisms: dinoflagellates⁴²; chlorophytic algae⁴³; hypermastigote and other flagellates⁴⁴⁻⁴⁶; amoebae and amoeboflagellates⁴⁷; ciliates^{48,49}; flagellated fungi⁵⁰; and cellular slime moulds⁵¹.

These steps include, for example, the origin of the nucleolar cycle; chromosomal organisation involving histone, non-histone proteins and chromatin RNA; the development of the chromosomal coiling cycle; the development of microtubule-mediated gene distribution mechanisms (including the origins of kinetochores, centrioles or spindle plaques, and spindle pole bodies); the origin of sex-determining mechanisms, sexual attraction systems, syngamy, nuclear fusion, synapsis, bivalent formation and reduction division. It is plausible to assume that the origin and stabilisation of these elaborate cell division mechanisms evolved in the group of organisms that still shows variations: the eukaryotic microorganisms. There has always been a general consensus among botanists and zoologists that plants and animals are descendants of these protists. We may assume then that mitosis followed by meiosis arose in protists sometime between 2 and 0.7×10^9 yr ago, after the transition to the oxygenic atmosphere. Because of their complexity these processes may have taken a long time to evolve; their

development must have preceded the origin of most multicellular eukaryotes. We therefore attribute the late appearance of metazoa, metaphyta and fungi to primarily biological rather than environmental factors.

It has recently been established that mitotic microtubule systems require low and regulated calcium ion concentrations for polymerisation of the component tubulin protein into tubules⁵². Thus, it is possible that the removal of calcium and its external deposition evolved primarily for the intracellular stabilisation of the mitotic apparatus and other somatic microtubules, thereby preadapting many organisms for the formation of protective and supportive calcium carbonate hard parts. This idea is supported by the observation that microtubules of the axopods of multi-chambered foraminiferans are involved in the calcium carbonate deposition of the shells⁵³. According to this idea, calcium carbonate depositional systems generally require intracellular interactions with microtubules. Calcium carbonate shell deposition could easily have originated in populations of protists and metazoan organisms that regulated intracellular calcium ion concentrations in carbonate-rich waters.

Environmental and biological factors

In summary we suggest the following sequence of coupled environmental and evolutionary changes during the Archean and Proterozoic eras: the origin and diversification of anaerobic bacterial life that developed many mechanisms of ultraviolet avoidance, tolerance, and protection; the origin of oxygen-eliminating photosynthesis in cyanobacteria (resembling their modern counterpart, *Oscillatoria limnetica*⁵⁷), microorganisms that utilised atmospheric ammonia, nitrogen, or reduced organic nitrogen as sources of nitrogen for protein synthesis. The evolution of physiologically modern blue-green algae led to the release of oxygen into the environment, primarily by blue-green algal mat communities. The existence of these communities is inferred from their lithified remains—Precambrian stromatolites. Photosynthetic oxygen production permitted the abiological formation of nitrites and nitrates as well as the accumulation of oxygen in the atmosphere. The use of nitrate and nitrite as still another mechanism of protection against ultraviolet irradiation may have led to the origin of nitrate reductive pathways as a source of cellular nitrogen which possibly were precursors of aerobic respiration^{37,38}. The evolution of major metabolic pathways included the origin of facultative and obligate aerobiosis in prokaryotes.

The diversification of prokaryotes was accompanied by the evolution of symbiotic interactions between groups of microorganisms including those that were ancestral to modern eukaryotes. The rise of modern aerobic eukaryotes, including many lines of multicellular forms, then followed only after the evolution of microtubule-utilising mitosis and meiosis. The origin of mitotic and eventually meiotic systems occurred in various lines of eukaryotic protists. The speciation events that produced major groups of multicellular eukaryotes (plants, animals, and fungi) occurred in an oxygenic world⁵⁵.

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Evaluation of six short term tests for detecting organic chemical carcinogens and recommendations for their use

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Six short term tests for detecting carcinogenicity have been evaluated using 120 compounds, of which half were carcinogens and the rest non-carcinogens. The results obtained indicate that the Ames test and a "cell transformation" assay are both sufficiently sensitive to carcinogenicity, or the lack of it, in the compounds studied to enable them to be employed for detecting potential carcinogens. The consequences of using short term tests under various screening conditions have been explored. In order to have confidence in the results obtained for new or previously untested compounds it is important to use such tests in a carefully controlled manner.

Two methods are commonly used for predicting the potential human carcinogenicity of an organic chemical. The first is to examine its chemical structure for similarities to those of known human or experimental carcinogens, and the second, which usually follows from a positive correlation in the first, is to undertake animal carcinogenicity studies. Although this approach has revealed many new carcinogens it is time consuming and expensive, and further, it tends to identify new analogues of established carcinogens rather than carcinogens of novel structure. It is, therefore, not surprising that clinical observations or epidemiological evidence have sometimes given the first indication that a particular chemical is a human carcinogen. Such cases, as exemplified by the increased incidence of bladder cancer among both benzidine¹ and β -naphthylamine² workers, have usually been limited to small groups of people in the particular industry or activity concerned, and although each one has been a serious individual hazard they collectively contribute only a small part to the total cancer burden of the population at large.

It has been suggested by several scientists in this field that a significant proportion of the 'spontaneous' human cancer in-

cidence is dependent on environmental factors, one of which may be the presence of both natural and contaminant carcinogens in the environment^{3,4}; however, to test every environmental and industrial chemical for carcinogenicity by conventional methods would clearly be impossible within the foreseeable future. It is mainly for this reason that attempts are being made to develop short term tests for potential carcinogenicity.

A variety of short term tests have been described (see ref. 5 and refs therein), but only one, the Ames test (ref. 6 and refs therein), has been evaluated in any detail. We therefore undertook an evaluation of a variety of established and novel short term tests to determine their strengths and limitations and to decide if any one test, or a combination of them, could form the basis of a screen. In addition, we needed to consider how such a screen could most effectively be used to avoid the development of unrecognised potential human carcinogens in the future and also to detect any unsuspected carcinogens which might still be in use.

Evaluation of the chosen short term tests

Ten short term tests have been evaluated. The choice of tests was decided partly on empirical grounds and partly by the need to work within the resources made available for this study. Thus, the exclusion of other available tests from this study does not mean that they should automatically be excluded from any future carcinogen screens. A brief evaluation of the iodine⁸ and the acridine⁹ colour tests, the transplacental blastomagenesis test¹⁰ and the piperidine alkylation test¹¹ was sufficient to establish that they were unsuitable for our purposes, and consequently, they were not pursued further.

The main evaluation was of the six tests described below and was carried out using 120 organic chemicals, 58 of which are known human or animal carcinogens, the remaining being putative non-carcinogens. The difficulties implicit in such classification have been commented on elsewhere⁵ and the guidelines developed for this study will be described in detail later. The compounds about

Table 1 Results of six short-term tests for carcinogenicity

	Polycyclics	Arylamines	Alkylating agents	Miscellaneous	Total
1 Ames test	95	94	83	94	93
2 Cell transformation	95	97	94	92	94
3 Rabin's test	65	76	61	73	71
4 Subcutaneous implants	88	60	25	81	68
5 Sebaceous gland suppression	90	62	67	57	65
6 Tetrazolium reduction	50	56	39	67	57

120 compounds (carcinogens and non-carcinogens) were tested. The figures refer to the percentage of accurate predictions made by each test for individual classes of compounds and for the total group.

which any doubt existed, such as trimethyl phosphate were, however, few and a change of their classification would not substantially affect the overall results.

The compounds were chosen to represent a wide range of carcinogens and non-carcinogens and have been somewhat arbitrarily subdivided into chemical classes. Where possible, structurally related carcinogen and non-carcinogen pairs were included in the study. The compounds were coded and each was tested as a solution in either dimethyl sulphoxide (DMSO) or water without operator knowledge of their individual biological activity. Each compound was tested once in each of the test systems and no attempt was made to optimise the test conditions for a particular compound, nor to determine the reproducibility of individual results. A detailed presentation of this study will be made at a later date, but the following is a summary of the methods employed and the results obtained.

● **Ames test.** Compounds were tested using four strains of *Salmonella typhimurium* (TA 1535, 1538, 98 and 100) in the plate incorporation assay of Ames⁶. A rat liver microsomal preparation (S-9 fraction: cofactor, 1:3) was used⁶.

● **Cell transformation.** A novel technique has been devised in which neonatal Syrian hamster kidney fibroblasts (BHK21/C13) and either human diploid lung fibroblasts (WI-38) or human liver cells (Chang) were exposed to five different doses of the test compounds *in vitro* in liquid tissue culture medium without serum. The S-9 mix of the Ames test⁶ was included in the culture medium to aid in the metabolism of the test compound. To assess survival following exposure to a compound a small number of cells from each treated sample were grown in liquid medium and colonies counted after 6–8 d incubation. A dose–response curve for survival was constructed and the LD₅₀ calculated. The remaining cells were transferred to semi-solid agar which permitted the growth of transformed cells¹². A dose–response curve for transformation was constructed and the number of transformed colonies at the LD₅₀ dose calculated. A 2.5 times increase in colonies over controls was regarded as positive. Although this test is referred to as cell transformation, growth in semi-solid agar is only one of the accepted criteria for cell transformation.

● **Rabin's test.** The loss of ribosomes from isolated rat liver rough endoplasmic reticulum (RER) following incubation with potent carcinogens *in vitro*, as first described by Williams and Rabin¹³, has been quantitatively monitored by radio-tracer techniques¹⁴. An increase (10% or greater) in degranulation of the RER by the test compound, as compared with negative controls, was taken to indicate a positive result.

● **Implant test.** A novel technique has been developed based on the histological assessment of the fibrous capsule and surrounding tissues which develop three months after surgical implantation in mice of Millipore filter disks overlain with a gelatinous suspension of the test compound.

The histological appearance of the capsule surrounding each implant was scored on a scale between 1 (low) and 5 (high) based on the appearance of lesions thought to be indicative of neoplasia. The mean scores from control implants containing only DMSO and gelatin were then compared with those from the test implants. Compounds causing a statistically significant increase in the mean score were taken as positive in the test.

● **Sebaceous gland test.** Bock and Mund have demonstrated that the sebaceous glands of mouse skin are sensitive to the topical

application of carcinogens¹⁵. Test chemicals were applied directly to mouse skin and those causing a statistically significant decrease in the ratio of sebaceous glands to hair follicles were taken to be positive.

● **Tetrazolium reduction test.** The test was based on that described by Iversen and Evensen¹⁶. Samples of mouse skin which had been exposed to the test compound *in vivo* were incubated in aqueous solutions of tetrazolium red. Statistically significant increases in the *in situ* biological reduction of the colourless tetrazolium compound to the coloured formazan were measured spectrophotometrically and taken to indicate a positive response for the test compound.

A summary of the results obtained in this study is given in Tables 1–3. An overall impression of the performance of each of the tests can be gained from the 'total' column of Table 1, which shows the percentage of correct predictions made by each test with the complete group of 120 compounds. It is evident that only the first two tests performed well. The remaining columns of Table 1 show how each test performed with the individual chemical classes of compounds. With the exceptions of the response of test 4 to alkylating agents and test 5 to polycyclic aromatic hydrocarbons, the predictive accuracy of each of the tests is essentially independent of the chemical class of the test compound. However, it is probable that a different study using different test compounds from those used here would give slightly different overall performance figures.

An alternative method of assessing the results produced by the various tests is shown in Table 2. For a test to be useful for either research or screening purposes it must be capable of detecting a

Table 2 Results from the six short term tests expressed as the percentage of accurate predictions for 58 carcinogens and 62 non-carcinogens

CARCINOGENS		
1 Ames test		91
2 Cell transformation		91
3 Rabin's test		71
4 Subcutaneous implants		37
5 Sebaceous gland suppression		67
6 Tetrazolium reduction		40
NON-CARCINOGENS		
1 Ames test		93
2 Cell transformation		97
3 Rabin's test		71
4 Subcutaneous implants		95
5 Sebaceous gland suppression		64
6 Tetrazolium reduction		73

Table 3 Response of the six short term tests to eight carcinogen and non-carcinogen pairs

Test compound	Ames test	Cell transformation	Rabin's test	Subcutaneous implants	Sebaceous gland suppression	Tetrazolium reduction	Animal carcinogenicity
4-Nitroquinoline- <i>N</i> -oxide	+	+	+	+	+	+	+
3-Methyl-4-nitroquinoline- <i>N</i> -oxide	—	—	—	—	—	—	—
Benzidine	+	+	+	—	+	—	+
3, 3', 5, 5'-Tetramethylbenzidine	—	—	—	—	—	—	—
2-Acetylaminofluorene	+	+	+	—	—	—	+
4-Acetylaminofluorene	—	—	+	*	*	*	—
9, 10-Dimethylantracene	+	+	—	+	+	+	+
Anthracene	—	—	+	—	—	—	—
Dimethylcarbamoyl chloride	+	+	—	+	+	—	+
Dimethylformamide	—	—	—	—	—	—	—
1-Fluoro-2, 4-dinitrobenzene	+	+	—	+	+	—	+
1, 3-Dinitrobenzene	—	—	—	—	+	—	—
β -Naphthylamine	+	—	+	—	+	+	+
α -Naphthylamine	—	—	—	—	—	—	—
Nitrosolic acid	+	+	+	—	—	—	+
Diphenylnitrosamine	—	—	+	—	+	+	—
Number of pairs correctly identified	8	7	2	4	5	3	

* Not tested

high proportion of any carcinogens which may be present in a group of compounds, and in doing so generate as small a number of false positives as possible (a false positive is defined here as a positive result obtained in a test system with a compound which has been tested for carcinogenicity in animals and found to be negative). Table 2, therefore, records the percentage of correct predictions made by each of the tests in the two complete subgroups of carcinogens and non-carcinogens and it is clear that only the first two tests possess the required balance of response. Obviously, the lower the detection rate of a test to non-carcinogens, the higher the number of false positives produced. The remaining four tests were generally less reliable. For example, tests 3 and 5 correctly identified 71% and 67% respectively of the test carcinogens but in doing so they generated a high proportion of false positives (29% and 36% respectively). This would therefore reduce the reliance that could be placed on a positive result generated by either of these tests for a compound of unknown biological activity. In contrast, test 4 correctly identified only 37% of the test carcinogens and generated a low proportion of false positives (5%). While these results make tests 3–5 of little value in isolation for screening purposes they may be of value when used in combination with other tests or for assessing the likely carcinogenic potency of a potential carcinogen. Test 6 performed poorly in all respects and is, therefore, of no general value.

The results shown in Tables 1 and 2 clearly establish that the Ames test (test 1) and the cell transformation assay (test 2) are both able to detect a high percentage of a wide range of carcinogens while also generating an acceptably low level of false positives. The usefulness of these two tests is further underlined by the selected results shown in Table 3. These illustrate that with an exception, that of test 2 with the naphthylamines, both are capable of correctly distinguishing between the eight structurally related carcinogen and non-carcinogen pairs used in this study. It is of importance to any screening programme that might use either of these tests that they have been shown to be sufficiently sensitive to register, for example, the weak carcinogen 9,10-dimethylantracene as positive while finding the parent compound anthracene, a non-carcinogen, negative. This structural sensitivity should also enable these tests to be employed at the research level to monitor the development of future series or classes of chemicals which possess structural similarities to known carcinogens. However, the ability of a test to distinguish correctly between carcinogens and non-carcinogens of the particular chemical class of compounds being studied will need to be established for each such class.

If the responses of the first two tests are compared it is found that they agree with each other in correctly predicting the activity of 106 of the 120 compounds (88%). In contrast, they both disagree with

long term evidence in only two cases, those of diethylstilboestrol and vinyl chloride. (Vinyl chloride produces a reproducible positive result in the Ames test when tested as a gas rather than as a solution in DMSO.) The remaining 12 compounds about which the tests disagree represent six established carcinogens and six putative non-carcinogens. Thus, if the results of these two tests are combined and a positive response from either system taken as an indication of activity they register all of the known carcinogens used in this study as positive, with the exceptions of diethylstilboestrol and vinyl chloride, and at the same time generate six false positive results.

Use of short term tests in screening programmes

The main appeal of short term tests is that they seem to offer a method of rapidly searching a group of compounds for potential carcinogens in order that priorities may be set for conventional long term studies. However, the drawback to using even relatively reliable tests for such purposes is that the proportion of positives produced which would be expected to be false may become very large. It is not possible from the present study to accurately estimate this proportion, but the model shown in Table 4 illustrates the nature of the problem which may be encountered. For example, in the model the effect of using a test which was known to

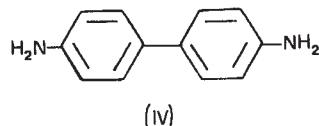
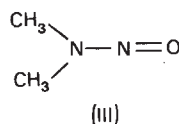
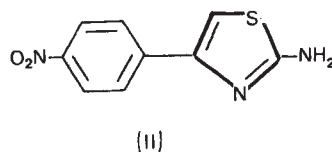
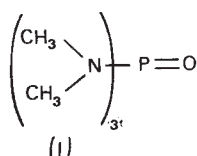
Table 4 Model of the way in which a screening test would operate under various conditions

Test accuracy	Proportion of carcinogens			
	0.1 %	1 %	5 %	50 %
99 %	11* (91 %)†	20 (50 %)	59 (16 %)	500 (1 %)
97 %	31 (97 %)	39 (75 %)	77 (37 %)	500 (3 %)
95 %	51 (98 %)	59 (84 %)	95 (50 %)	500 (5 %)
90 %	101 (99 %)	108 (92 %)	140 (68 %)	500 (10 %)

Positive results generated* for a group of 1,000 randomly chosen chemicals containing various proportions of carcinogens and using tests of various accuracies. The percentage value † refers to the proportion of these positives which would be expected to be false. This model is subject to the following assumptions being made. First, that the test accuracies for carcinogens and non-carcinogens are the same, and second, that results are reproducible and therefore not associated with experimental error. Finally, it has been assumed that when extrapolating data from Table 1 to the model situation of Table 4 the ratio of positive and negative results in the two categories (carcinogens and non-carcinogens) remains constant.

be 90% accurate to screen an arbitrarily selected sample of 1,000 chemicals containing 10 carcinogens (1%) would be to generate 108 positive results of which only nine would be carcinogens. There would, therefore, be only an 8% chance that a compound which was positive in the test would be an animal carcinogen. Conversely, in the present example, the probability that a compound which gave a negative result in the test would be a non-carcinogen would be greater than 99%.

Even if the test systems were to be further improved it is inevitable that there will always remain a finite level of false positives (and false negatives) due to the unavoidable existence of differences in enzyme levels and activities, biological absorption and distribution, DNA repair mechanisms and so on between the test system and the whole animal. Thus, faced with the certainty that any screening programme will generate equivocal positive results it is important to establish in advance how these results would be handled. One approach would be to submit each compound that had been shown to be positive in the screen to conventional animal tests. Alternatively, the future use of all such compounds could be halted. Both of these approaches are drastic and would probably result in unjustifiable economic and social disruption. A more realistic approach to this problem might be to extrapolate existing knowledge of the molecular structures and physicochemical properties of the many established animal carcinogens either to preselect chemicals for submission to the screen or to decide which of the compounds that had shown a positive result in the screen were most worthy of further evaluation. It seems likely that compound preselection would enable a larger proportion of any previously unrecognised environmental carcinogens to be characterised and controlled in the immediate future, and this approach has been developed below.



To act as an effective pre-screen the drawing of structural analogies between untested compounds and established carcinogens must be conducted quite liberally. Thus, it would be necessary to regard all derivatives of hydrazine, all nitrosamines, all compounds containing a polycyclic aromatic nucleus, and so on as candidates for the screen. Moreover, the term 'derivatives' as

used here would have to embrace relatively distant analogues of the various reference carcinogens. For example, any such pre-screen should be so conceived that it would have selected for testing the two recently described carcinogens hexamethylphosphoramide¹⁷ (I) (HMPA) and the aminothiazole¹⁸ (II) based on their structural resemblance to the reference carcinogens dimethylnitrosamine (III) and benzidine (IV) respectively.

Having selected various groups of compounds they should be tested in the presence of the structurally appropriate positive and negative controls. If, for example, compound (II) had been selected for testing it would be prudent to evaluate it in a test system that could, at the same time, be shown to respond correctly to the control pair of benzidine and 3,3',5,5'-tetramethylbenzidine (see Table 3). Greater weight could be given to the result obtained for (II) in such chemical class controlled conditions than had it been generated in isolation.

Two further considerations argue strongly in favour of the use of class-specific as well as test-specific control pairs. The first is that if members of the chosen control pair are correctly distinguished by the test it increases the probability that the physical, chemical and enzymic conditions of the test system have been optimised for the particular class of compound being studied, and the second is that it would be unwise at the present time to assume that any test is equally sensitive to carcinogenicity within all classes of present or future carcinogens. It would, for example, be of little value to monitor analogues of the novel carcinogen HMPA (I) with a test that was incapable of registering HMPA itself as positive.

The main objection to the above approach is that it implies that sufficient is already known of the broad structural features of all established and possible future carcinogens. Such may not be the case and it would therefore be necessary, in addition, to select and screen representatives of new or previously untested chemical classes. If several analogues of such a class were to prove positive in the screen, or if the level of human exposure to such a positive compound was high, the undertaking of conventional long-term testing would be appropriate.

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letters to nature

Continuum variability in Nova Cygni 1975

ONE of the more unusual aspects of Nova Cygni 1975 (V1500 Cyg) has been the presence of nearly periodic (~ 3.2 h) photometric variations beginning early in the decline (discovered by Tempesti¹ on September 9-10, 1975) and lasting up to the present. In spite of the excellent coverage available, the cause of this photometric activity is uncertain. In particular, broadband photometry has been unable to discriminate between continuum and emission line variations, which is necessary because the relative importance of

continuum and line emission has changed as the nova has developed. The relationship of this variation to classical transition phase oscillations in novae is also uncertain. We have obtained very low noise ($s/n \sim 100$) time resolved (~ 11 min per observation, extending over 87 min) 0.8-Å resolution scans of the [OIII] $\lambda\lambda$ 4959, 5,007-Å complex in Nova Cygni starting at 0943 UT on August 21, 1976, using a multichannel Reticon (Vogt and Tull, in preparation) at the coude focus of the 2.7-m telescope at the McDonald Observatory. The scans, which are shown in Fig. 1, establish the stability (profile variations $< 5\%$) of this feature. We

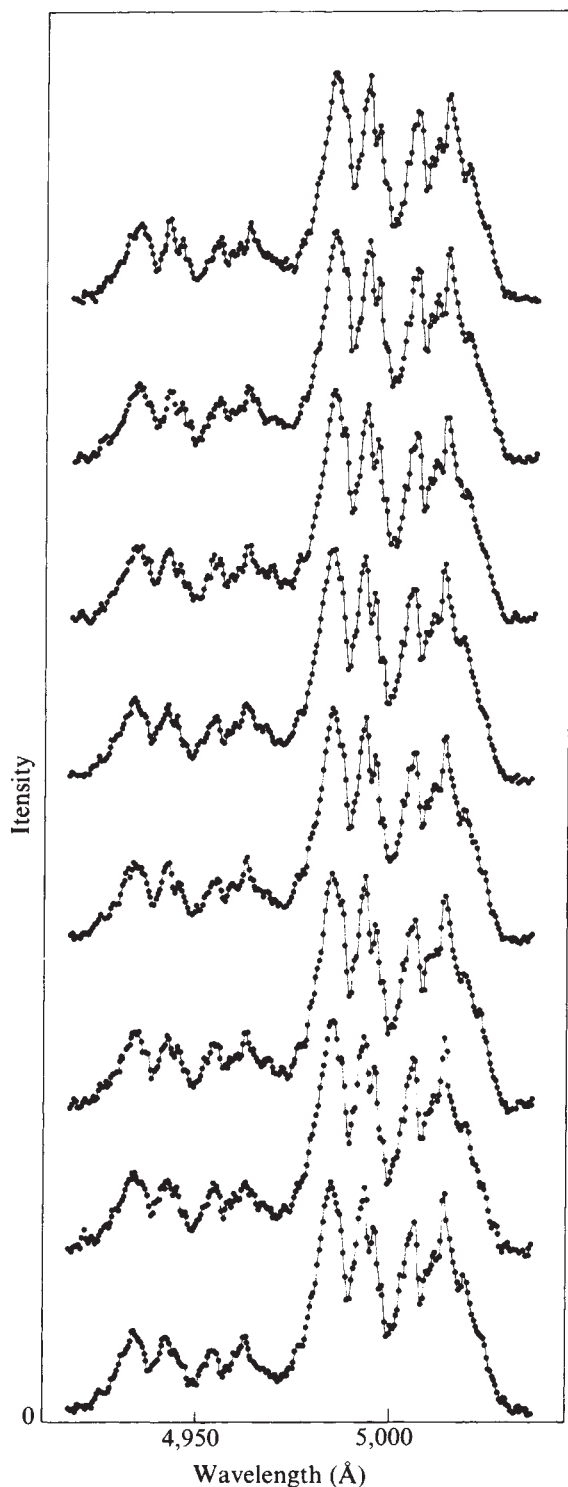


Fig. 1 Scans of the [OIII] 4959, 5,007-Å emission feature in Nova Cygni made on August 21, 1976 with a Reticon at the coude focus of the McDonald Observatory 2.7-m telescope. The scans, from top to bottom, ended at 0946, 0959, 1011, 1022, 1035, 1045, 1056 and 1107 (UT).

infer the constancy of the [OIII] $\lambda\lambda$ 4959, 5,007-Å emission feature and further infer the constancy of the other forbidden lines.

The observations being spectroscopic do not exclude the possibility that the strength of the [OIII] emission feature as a whole is varying. A 3.2-h variation of the emission feature as a whole is very unlikely, however, because of the size of the nova envelope, which at the time of observation was at least 2 light days across.

The emission from a line excited by collisions, such as the [OIII] line, is proportional to $N_e N_A$ where N_e is the electron density and N_A is the density of the atom under consideration. The Balmer lines are formed by capture-cascade whose emission is proportional to $N_e N_H$, $\sim N_e^2$ where N_H is the proton density, and we have assumed that hydrogen is the dominant electron donor. Both the collisionally excited and the capture-cascade lines are thus sensitive to the density, and so we may infer that the Balmer series is also constant at present.

Thus only the continuum remains as a possible source of the variation, in spite of the fact that it is a minor contributor to the flux in the Johnson V filter. To check this we undertook two nights of conventional differential photometry using a pulse-counting RCA 8850 (bi-alkali photocathode) on the McDonald 76-cm telescope. BD +47 3348 was the comparison star. A Stromgren y filter, whose flux is dominated by the continuum, was used together with a Crawford H β wide filter, whose flux is mainly from [OIII] $\lambda\lambda$ 4959, 5,007 Å and H β . The results are shown in Fig. 2 which demonstrates that the continuum is highly variable while the total flux in [OIII] and H β is nearly constant. The small variations in the H β wide filter are consistent with an origin in the continuum. We see that the 3.2-h variability of the nova originates in the continuum.

The contribution of the emission lines to the total flux in each of the broad band filters U, B, V and R varies considerably from one filter to another. Figure 3 shows a low resolution (4 Å) scan of the spectrum of the nova made on August 21, 1976, which has been corrected for a mean instrumental response function, and the UBV system functions given in Johnson². In the U filter the emission lines and continuum make comparable contributions to the total flux. The flux in the R filter, which has peak transmission at 7,000 Å (not shown in Fig. 3), is dominated by the H α , [NII] 6,548, 6,583 Å emission feature. We expect the broadband variability of the nova to be most marked in U and almost absent in R.

It is clear that interpretation of the broadband colours of the nova must allow for the constant emission lines. We cannot be sure how long the emission lines have been constant. A light travel time argument suggests that they must have settled down about the end of September 1975—a month after the nova outburst, when the nova envelope would have been at least 4 light hours across. Jeffers and Weller³ and Campbell⁴ report that in September 1975 the emission line strengths were varying so that during the first month or so after outburst it seems likely that both the continuum and emission lines were varying.

The most fruitful photometric observations of the nova should use filters chosen to exclude the constant emission lines and concentrate on the variable continuum. The Stromgren y filter is an example. Low resolution scans of the nova made by one of us

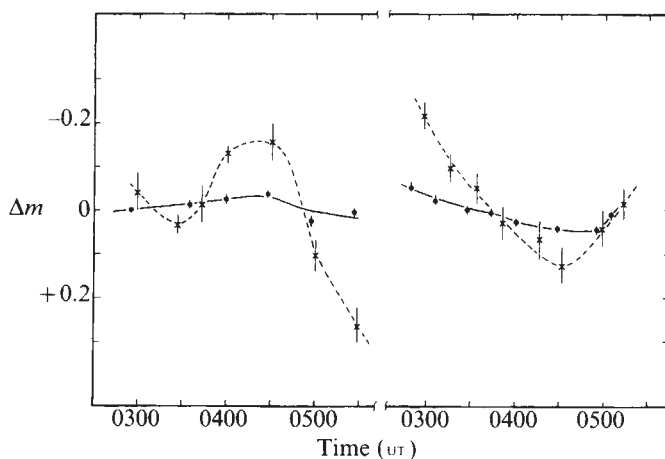


Fig. 2 Photometric observations of Nova Cygni made with a Stromgren y filter (---) and a Crawford H β wide filter (—). The flux in the y filter is mainly from the continuum, in the H β wide filter it is mainly from the [OIII] 4959, 5,007-Å and H β emission lines. The left-hand curves are for August 22, 1976 and the right-hand curves for August 23, 1976.

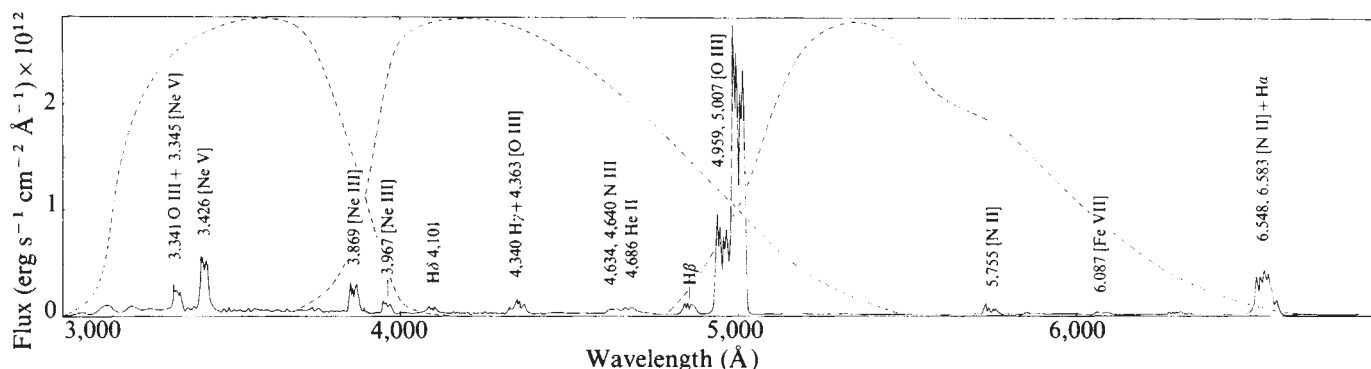


Fig. 3 Spectrum of Nova Cygni obtained on August 21, 1976 with the McDonald Observatory 2.7-m telescope. The absolute calibration (uncorrected for interstellar reddening) was obtained from our continuum observations and is uncertain by 10% because of the continuum variation. The response functions of the Johnson UBV filters are shown.

(J.W.) at regular intervals since its outburst show that since June 1976 there have been no major changes of the relative strengths of the emission lines. Thus, for the present, the scan of the nova spectrum in Fig. 3 should be a good guide for selecting other filters for observing the nova continuum.

We conclude that the continuum variations must originate in the central object rather than in localised regions of the envelope because of the stability of the emission line profiles and light travel time arguments. The importance of further continuum observations cannot be overstated. The object is presently > 7 mag above minimum light in the continuum, indicating that surface activity has not ceased. Further observations aimed at measuring the colour over the phase and determining accurate periods are essential to find out more about the nature of the variation and the central object.

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Spectral analysis and the astronomical theory of climatic change

THE astronomical theory¹ attributes long term changes in climate to changes in the Earth's orbital geometry. This theory has become increasingly accepted in recent years. To this acceptance, workers associated with the CLIMAP project have added exceptionally strong support² using spectral analysis of deep-sea core data. They have linked periodicities in the Earth's orbital parameters with periodicities in deep-sea core parameters related to sea-surface temperature, ice volume and oceanic salinity. Their argument rests on the assumption of a linear response between input (radiation changes caused by changes in orbital geometry) and output (the climate-dependent parameters mentioned above). At the same time they have attributed the anomalously high variance associated with the longest periodicity ($\sim 100,000$ yr) to a nonlinear response. There is no contradiction here because the response function, the climate 'black box', most probably has both linear and nonlinear components. I examine here the question of nonlinear response in more detail.

The input spectra given by Hays *et al.*² show strong peaks at periods of $\sim 41,000$ yr (associated with obliquity), and 23,000 and 19,000 yr (associated with precession). There is also a fundamental periodicity of $\sim 100,000$ yr associated with eccentricity, but this does not appear in the spectral record because eccentricity acts mainly to modulate the amplitude of the precession 'cycle'. The output spectra of Hays *et al.*² show strong peaks at $\sim 100,000$, 43,000 and 24,000 yr. There is also a peak at 19,000 yr in the $\delta^{18}\text{O}$ record (related to ice-volume changes). Most of the variance is contained in the 100,000-yr peak (~ 50 per cent), contrasting strongly with the lack of power at this period in the input signal. Hays *et al.*² convincingly link the 43,000- and 24,000-yr periodicities with obliquity and precession respectively.

Consider the effect of a nonlinear response mechanism on a simple input signal and on an amplitude modulated input signal. Firstly, as Gray³ has shown in a spectral analysis of precipitation data, an input signal with spectral peaks at frequencies ω_1 and ω_2 can produce an output signal with peaks at frequencies $\omega_1 + \omega_2$ and $\omega_1 - \omega_2$. A simple mathematical analysis can show how this can occur. Consider an input $X(t)$

$$X(t) = \sin \omega_1 t + \alpha \sin \omega_2 t \quad (1)$$

which is operated on by a nonlinear response function producing an output $Y(t)$

$$Y(t) = [X(t)]^2 + a[X(t)] \quad (2)$$

Note that $Y(t)$ contains a linear term $a[X(t)]$. Substituting equation (1) into equation (2)

$$Y(t) = a \sin \omega_1 t + \alpha a \sin \omega_2 t - \frac{1}{2} \cos 2\omega_1 t - \frac{\alpha^2}{2} \cos 2\omega_2 t + \alpha \cos(\omega_1 - \omega_2)t - \alpha \cos(\omega_1 + \omega_2)t + \frac{1 + \alpha^2}{2} \quad (3)$$

$Y(t)$ contains power at both composite frequencies $\omega_1 + \omega_2$ and $\omega_1 - \omega_2$. Power at the input frequencies ω_1 and ω_2 is only transmitted through the linear part of the response function.

Secondly, consider an amplitude modulated periodic signal of the form

$$F(t) = (1 + \beta \sin \omega_3 t) \sin \omega_4 t$$

where ω_4 is the frequency of the basic signal and ω_3 is the frequency of amplitude modulation. Spectral analysis of this signal produces a major peak at ω_4 with minor peaks at $\omega_4 \pm \omega_3$, but no peak at the amplitude modulating frequency. If, however, this signal is operated on by a simple nonlinear response function

$$Z(t) = [F(t)]^2$$

the spectrum of the output, $Z(t)$, has a dominant peak at the amplitude modulating frequency (and, additionally, an array of

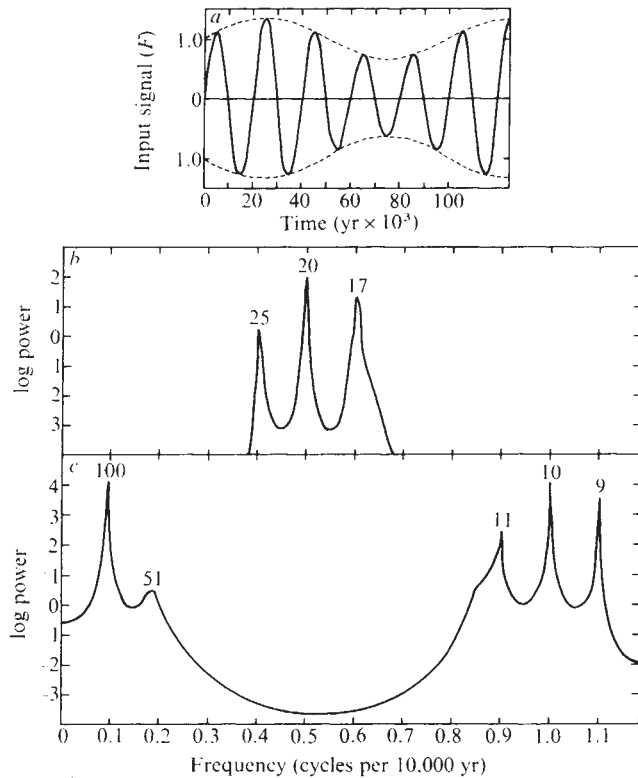


Fig. 1 Amplitude-modulated sine wave signal (a) and MESA spectra of signal (b) and signal-squared (c). The chosen periods of 20,000 and 100,000 yr approximate those of the orbital parameters. For both spectra the Nyquist frequency is 2.5 cycles/10,000 yr (4,000-yr period). A minor peak at a frequency of 1.2 cycles/10,000 yr lies outside the range plotted in the lower spectrum. The numbers above the peaks give periods in thousands of years.

peaks centred around $2\omega_4$). These results are illustrated in Fig. 1 which shows a signal of the type $F(t)$, its Maximum Entropy Spectral Analysis (MESA) spectrum, and the MESA spectrum of $Z(t) = [F(t)]^2$.

The results of Hays *et al.*² can be examined in the light of these simple results, bearing in mind that the real response function must be considerably more complex than the extremely simple ones considered above. The orbital input spectra show a split peak near the precession period of 21,000 yr (Table 4 of Hays *et al.*² gives peaks at periods of 23,100 and 18,800 yr). Nonlinear interaction between these two frequencies could be expected to give an output signal with period 101,000 yr (the $\omega_1 - \omega_2$ term in equation (3)). In addition to this effect, nonlinear response to amplitude modulation of the precession signal will produce a spectral peak at the frequency of amplitude modulation; namely at the eccentricity period of $\sim 100,000$ yr. These two possibilities are closely related if the precession peak splitting is caused by amplitude modulation: the magnitude of the $\omega_1 - \omega_2$ period, 101,000 yr, is remarkably close to the eccentricity period and so admits this possibility.

Either individually, or together, these two mechanisms both lead to strong output power near a period of 100,000 yr, with little or no input power at this period. The data of Hays *et al.*² also show significant power at periods corresponding to precession and obliquity, so the climate response function cannot be wholly nonlinear. The indication is that the response function has both linear and nonlinear parts of approximately equal importance.

This analysis raises two further questions; why is the response nonlinear and why is it that other nonlinear interaction frequencies (such as the $\omega_1 + \omega_2$ term in equation (3)) are not evident in the deep-sea core record? Hays *et al.*² suggest that nonlinearity may arise from a difference in response times for ice build up and decay at the start and end of glacial periods. An alternative answer lies in the albedo-radiation input feedback

mechanism. Climatic sensitivity to energy inputs can be examined usefully using the radiation-balance equation⁴

$$\sigma T^4 = \frac{S}{4}(1 - A)$$

where σ is the Stefan-Boltzmann constant, S is the solar constant, A is the global-mean albedo and T is the radiative-equilibrium temperature of the Earth. In terms of perturbations from an equilibrium temperature T_0 at S_0 and σ_0 one obtains

$$T - T_0 \approx \frac{T_0}{4} \left(\frac{\delta S}{S_0} - \frac{\delta A}{1 - A_0} \right)$$

where $\delta S = S - S_0$ and $\delta A = A - A_0$. Any feedback mechanism behaving like

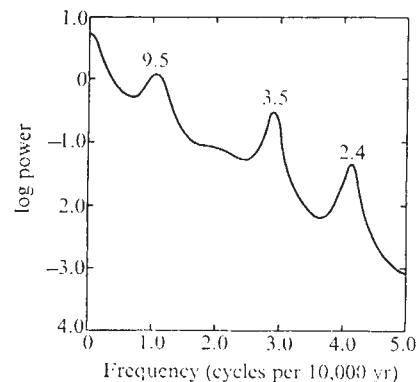
$$\delta A = C_1 \delta S + C_2 (\delta S)^2$$

will yield the form of nonlinear response function considered earlier. There is little doubt that changes in albedo will occur if S is changed (one argument proposed is that lower S produces lower temperatures, more snow cover, and lower global mean albedo); and it seems certain that the albedo-solar constant (or albedo-temperature) feedback will not be linear.

This argument primarily illustrates how feedback may be related to, or cause, nonlinear behaviour of the climate response function. Many feedback mechanisms have been proposed, and most probably more than one operates at any one time. The complexity of the situation can be gauged by the fact that both positive and negative feedbacks between T and A (or between S and A) have been proposed. Deterministic climate models attempt to include important feedback effects, and a crucial test for such models is that they should produce realistic output spectra from correct orbital input data. Since many models are quasi-linear, nonlinearity must be 'built in' through feedback mechanisms.

The absence of other nonlinear interaction frequencies in the deep-sea core spectra may be a result of bioturbation or of the complexity of the climate response function compared with the simple models used here. Furthermore, the response function will differ for each output variable considered, and one need not expect different output variables to show identical spectra (as is indeed the case). One might expect, therefore, that some climate-dependent variables would show other nonlinear interaction frequencies. The $\omega_1 + \omega_2$ frequency corresponding to the 23,100- and 18,800-yr precession peaks gives a period of 10,400 yr. A similar periodicity arises from the amplitude modulation effect (see Fig. 1). This period occurs in the ^{14}C dating anomaly, but the link between this and climate is rather indirect. A period of

Fig. 2 MESA spectrum of oxygen isotope data from Iowa speleothems. The spectrum is a low-resolution spectrum (11 lags, 78 data points), and the data were neither detrended nor prewhitened. The Nyquist frequency is 5 cycles/10,000 yr. Numbers above peaks give periods in thousands of years.



~10,000yr is also evident in the oxygen isotope record from Iowa speleothems⁵. Such isotopic changes have been directly linked to climatic change by a number of workers⁶⁻⁸. A MESA spectrum of Iowa speleothem data spanning the period 6,000–83,000 b.p. (Fig. 2; data supplied by Harmon (personal communication)) has peaks at periods of 2,400, 3,500 and 9,500yr. The peak at ~2,400yr is interesting since a similar period has been detected in the isotopic variations of the Camp Century ice core⁹, and in the frequency of periods of glacial activity in the Holocene¹⁰⁻¹². The dominant peak (significance of MESA spectral peaks being proportional to the area under the peak) is, however, at 9,500yr which may correspond to the $\omega_1 + \omega_2$ nonlinear interaction discussed above.

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Upper-atmosphere zonal winds from satellite orbit analysis

By analysing the changes in the orbital inclination i of a suitable satellite, it is possible to determine zonal (west-to-east) winds in the Earth's atmosphere at heights near that of the satellite's perigee. These values of wind speed are usually averaged over latitudes between 0 and about $0.5i$; often the values are also averaged in local time, although some recent results range over only a few hours in local time.

We have critically reviewed all such results previously published, in the light of current standards of accuracy: some are accepted, some revised and some rejected. We have also

analysed a number of fresh orbits, giving a total of 44 values of the atmospheric rotation rate Λ (rev d⁻¹) from 35 satellites¹. These values, which apply at heights between 150 and 700 km, have been divided into three categories: (1) averaged in local time; (2) 'evening', defined as 1800–2400 local time; and (3) 'morning', defined as 0400–1200 local time.

The values of Λ are plotted against height in Fig. 1 for the three categories of local time. The evening values obtained from orbits analysed at the Royal Aircraft Establishment (RAE)¹⁻³ are indicated by upward-pointing triangles, the morning values^{1,3,4} by downward-pointing triangles, and the average values^{1,5,6} by black circles, all with unbroken 'error-bars'. We have also utilised results based on balloon satellites by Blum and Schuchardt⁷ and by Slowey⁸, and results using short-lived satellites by Forbes⁹; these are shown with broken lines as 'error bars'.

The solid curve in Fig. 1, through the points averaged over local time, shows that the rotation rate (in rev d⁻¹) increases from near 1.0 at 150 km to 1.3 near 350 km, corresponding to an average west-to-east wind of 120 ms⁻¹ at a representative latitude near 30°: the rotation rate then declines to 1.0 at 400 km, and probably to about 0.8 at greater heights. The upper broken curve shows that in the evening the wind is from west to east, increasing from about 50 ms⁻¹ at 150 km height, to a maximum of about 150 ms⁻¹ near 350 km; above that the data are sparse, but a decline to near zero by 600 km seems probable. The lower broken curve shows consistent east-to-west winds in the morning, of magnitude 50–100ms⁻¹ above 200 km.

It should be emphasised that these results refer to winds averaged over all conditions of solar activity and geomagnetic disturbance. The winds are sampled at latitudes from 0 to 55°, but with a bias towards the lower latitudes. The day-to-day behaviour of the atmosphere may vary widely from these norms: it is the strength—and the weakness—of the satellite orbit analysis that it gives an impression of the average atmosphere. Other techniques, such as radar back-scatter or measuring the motion of vapour trails released from rockets, can give much better resolution in local time and geographical location, but fail to indicate world-wide average values.

Still, it is possible to give some indication of how the wind speed varies with, or depends on, geomagnetic disturbances, solar activity and latitude. The values of Λ in Fig. 1, being averaged, are for quiet-to-medium geomagnetic conditions, but it is well known that winds are usually enhanced at the times of geomagnetic storms, when winds of up to 500 ms⁻¹ have been measured by vapour-trail methods¹⁰, radar back-scatter techniques¹¹ and orbit analysis⁹. We have looked for possible variations of Λ with solar activity and latitude, and

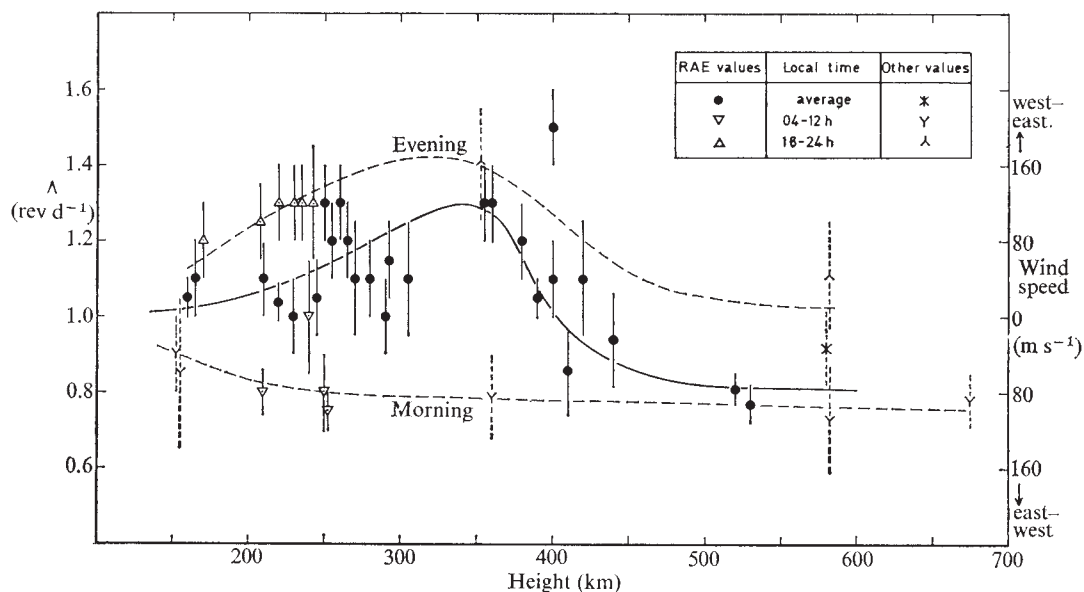


Fig. 1 Atmospheric rotation rate, Λ , against height.

we tentatively conclude from analysis of the values in Fig. 1 that Λ tends to be high when solar activity is low, and that, at heights above 350 km, Λ is larger in near-equatorial latitudes (0–25°) than at higher latitudes (25–50°).

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Radiocarbon dates and deglaciation of Rannoch Moor, Scotland

THE Loch Lomond Readvance affected large areas of the Scottish Highlands, and while the maximal extent of the readvance glaciers is now fairly well established^{1,2}, it is not yet known how long they took to decay³. Three radiocarbon dates have been obtained from a site on Rannoch Moor in the heart of the south-west Grampian Highlands that provide significant new information on the deglacial chronology of Scotland.

The site, Kingshouse 2, is situated in the north-western part of Rannoch Moor (grid ref. NN 282555) at an altitude of about 340 m. It is a small kettle hole within extensive deposits of hummocky moraine that are the product of a stagnating Loch Lomond Readvance ice cap. The subsurface contours of the basin were established by sounding the basin along a grid pattern, and the deepest point located accurately. Successive cores of 5 cm diameter were removed from the deepest point with a piston corer and subsequently analysed for pollen content. Material for radiocarbon assay was taken from four basal cores put down at the corners of a 30 cm square. The full stratigraphic sequence and pollen assemblage zones are shown in Fig. 1.

A date of $9,910 \pm 200$ BP (BIRM-724) was obtained from the peaty-gyttja horizon (lithostratigraphic unit 7) immediately above the contact with the underlying minerogenic sediments of lithostratigraphic unit 6. At that level, the pollen spectrum is characterised by very high percentages of *Juniperus* (over 75% total land pollen), with significant values for *Empetrum* and *Salix*. *Betula* counts are initially low, but there is a marked upward trend in the birch curve immediately above the dated horizon.

Lithostratigraphic unit 5 consists of fine sands with abundant remains of the moss *Racomitrium lanuginosum*, fragments of which provided a date of $10,290 \pm 180$ BP (BIRM-722). Immediately below the moss horizon, a thin layer of greenish-brown gyttja (lithostratigraphic unit 4) was discovered within the predominantly minerogenic sediments, and that organic layer yielded a date of $10,520 \pm 330$ BP (BIRM-723). A single pollen count was obtained from the organic deposit, the spectrum being dominated by *Empetrum* and *Juniperus*. The pollen data and the inwashed moss fragments suggest affinities with the *Racomitretum-Empetretum* and *Juniperetum nanae* associations which are common in parts of the western and northern Highlands today⁴. The former association occurs on block scree or bedrock with undeveloped soils, while the *Juniperetum nanae* represents a transitional association between

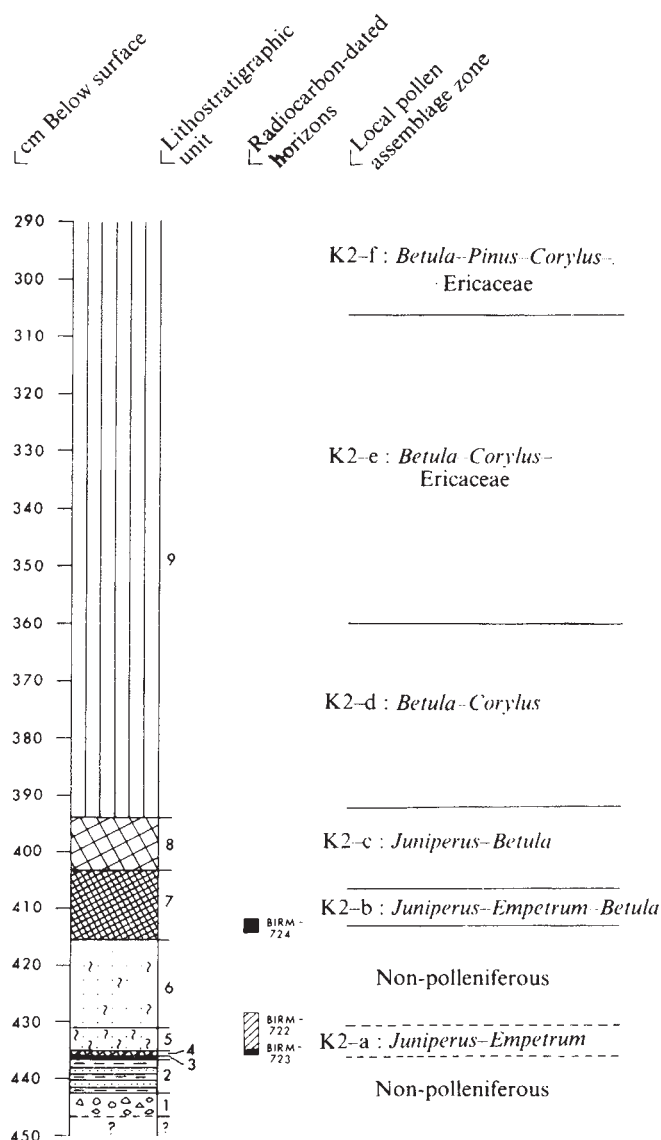


Fig. 1 Lithostratigraphy and biostratigraphy at deepest point of Kingshouse 2 kettle hole. 1, Gravel and coarse sand; 2, laminated silt and fine sand; 3, clay; 4, gyttja; 5, fine sand with abundant moss remains; 6, fine-medium sand with occasional moss fragments; 7, greenish-brown peaty gyttja; 8, light brown gyttja; 9, black telmatic peat with occasional fine roots.

subalpine scrub and low-alpine dwarf shrub heath. The evidence therefore implies that juniper and *Empetrum* heaths became established fairly soon after the deglaciation of Rannoch Moor while soils were still relatively immature, these heath communities being interspersed with patches of bare ground or ground covered by a grass or moss carpet.

The basal rhythmite sequence (lithostratigraphic unit 2) reflects variable conditions of sedimentation within the former lake basin. A maximum of 15 paired laminations was counted for an individual core, although whether these represent true varves is not certain. The lens of gyttja which yielded a pollen

Table 1 Kingshouse 2 radiocarbon dates

Laboratory reference	BIRM-723	BIRM-722	BIRM-724
Site reference	K2-436	K2-433	K2-410
Material	Gyttja	moss remains	Gyttja/lake mud
Weight (g)	54.95	12.40	110.83
Years BP	$10,520 \pm 330$	$10,290 \pm 180$	$9,910 \pm 200$
$\delta^{13}C_{PDB}(\text{‰})$	-22.37	-22.45	23.30

assemblage dominated by *Empetrum* and *Juniperus* indicates an early period of relative soil stability, but as the organic horizon is very thin and is overlain by more minerogenic sediments, it would seem that that period of stable soil conditions was short-lived. Since *Rhacomitrium lanuginosum* is a terrestrial moss species^{5,6}, and is therefore unlikely to have been growing around the basin littoral, the abundant fragments of the moss contained in the sands implies that the sediments are the product of increased surface erosion around the basin catchment. This in turn suggests that Rannoch Moor experienced marginal climatic conditions at that time.

The radiocarbon dates from Kingshouse 2 (Table 1) therefore relate to a very early stage in the evolution of the vegetation pattern after the severe climatic regime of the Loch Lomond Stadial, but before milder environmental conditions had become firmly established. In assessing the reliability of the dates, a number of points must be considered. First, there is a fairly large standard error associated with each of the age determinations, due mainly to the small amounts of material available for dating purposes. Secondly, whenever gyttja is used as a dating medium, there is always the risk of hard water error⁷, and while there are no outcrops of calcareous rock within the study area, the possibility cannot be excluded that small amounts of inert carbon may have become incorporated into the drift material from the complex metamorphic rocks that surround the Rannoch basin. Moreover, the small quantities of gyttja material used for dating and the nature of the gyttja itself precluded the use of NaOH in the pretreatment.

Conversely, the age determinations were made on both terrestrial moss and limnic sediments, the dates are internally consistent, and in no case does the $\delta^{13}\text{C}_{\text{PDB}}$ value lie outside the 'normal' range observed for wood, peat, gyttja and charcoal from European localities, that is, $\delta^{13}\text{C}_{\text{PDB}} = -25 \pm 5 \text{‰}$ (Table 1). Also checks have been run on all three dated samples. It was suspected that in the first preparation of BIRM-724 electron capture by electronegative impurities may have led to a slight diminution of the sample activity, and hence a duplicate preparation was undertaken. This second measurement of $9,910 \pm 200$ is preferred. A cross check was also made on BIRM-722, but in this case the same gas sample was counted twice. The two results are not significantly different at the 95% level (2σ), and thus a mean date of $10,290 \pm 180$ BP has been adopted. Finally, BIRM-723 was calculated by using two different values for the modern standard, but as the difference between the two assays was found to be minimal, the date of $10,520 \pm 330$ BP is considered acceptable (R.E.G. Williams, personal communication). Thus, as far as is possible to determine, the dates seem to be reliable within the limitations of the radiocarbon method.

We emphasise that these dates are minimal for deglaciation, for almost 10 cm of minerogenic sediment underlies the lowermost dated horizon, and residual ice may have remained in the basin for hundreds of years after ice had disappeared from the surrounding area⁸. The dates from Kingshouse 2 are the first to be published from the base of a kettle hole within the Loch Lomond Readvance limits, and if correct, provide a minimal date for the disappearance of ice from the vicinity of the site. Taken together, they suggest that Rannoch Moor became ice-free before 10,000 BP, perhaps even before 10,200 BP.

The Kingshouse 2 dates are important in a wider context. Previous studies have shown that the Rannoch basin was one of the major ice accumulation and dispersal centres in Scotland throughout the Pleistocene, and that it performed a similar function during the Loch Lomond Readvance². It seems likely that, because of the altitude and thick ice cover (over 400 m in places), the Rannoch basin would have been one of the last localities in Scotland to be deglaciated following the Loch Lomond Readvance. Hence, if the radiocarbon dates are correct they imply total deglaciation of Scotland some time before 10,000 BP.

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Model of material cycling in a closed ecosystem

ALTHOUGH ecosystem behaviour is ultimately determined by the combination of energy flow through the system and material cycling within it, remarkably little effort has been directed towards elucidating the effects of material cycling on ecosystem stability, noteworthy exceptions being the works of Ulanowicz¹, May², and Dudzik *et al.*³. Ulanowicz¹ and May² studied a model of a linear trophic chain in which both the biomass and energy fluxes between any two levels involved bilinear sums of the biomasses of all the species present and concluded that the system was stable only if the specific energy (that is, energy content per unit biomass) increased on ascending the trophic chain. Although producers are normally found to have a lower specific energy than consumers, there seems to be little or no significant difference between the specific energy of different consumer levels (see, for example, refs 4 and 5). The implication of the Ulanowicz–May model is thus that the stability of a trophic chain is a fragile property dependent on small differences in specific energy between levels. We believe that this result is due to a neglect of the distinctive nature of the dynamics of the decomposition of biological material to inorganic material, a viewpoint consistent with the results of Dudzik *et al.*³ who analysed a number of detailed models of nutrient cycles in both open and closed ecosystems. Here we propose an alternative model of a trophic chain, which suggests that the effect of material cycling is to stabilise the system.

We consider a linear chain of n trophic levels (see Fig. 1) and denote by x_i the number of mol of a given element in the i th trophic level. We follow Ulanowicz and May and model both predation and the uptake of inorganic material by quadratic terms of the Lotka–Volterra type. Our key assumption is that material in the i th level is converted directly to inorganic material at a rate $k_i x_i$. We denote by x_0 the number of mol of the element in the inorganic reservoir. The evolution of the ecosystem is then described by the equations

$$\dot{x}_0 = \sum_{i=1}^n k_i x_i - A_{01} x_0 x_1$$

$$\dot{x}_1 = -k_1 x_1 - A_{10} x_1 x_0 - A_{12} x_1 x_2$$

$$\vdots$$

$$\dot{x}_n = -k_n x_n - A_{n,n-1} x_n x_{n-1}$$
(1)

where the principle of conservation of elemental matter guarantees that $A_{ij} = -A_{ji}$ for all i, j . The model is most easily pictured when applied to carbon—the k_i are then respiration rate constants, A_{01} is a rate constant for photosynthesis, and x_0 is the standing crop of carbon in CO_2 . For other elements the terms $k_i x_i$ represent the combined effects of natural death and decomposer action, the implied assumptions being first, that the presence of decomposers does not signi-

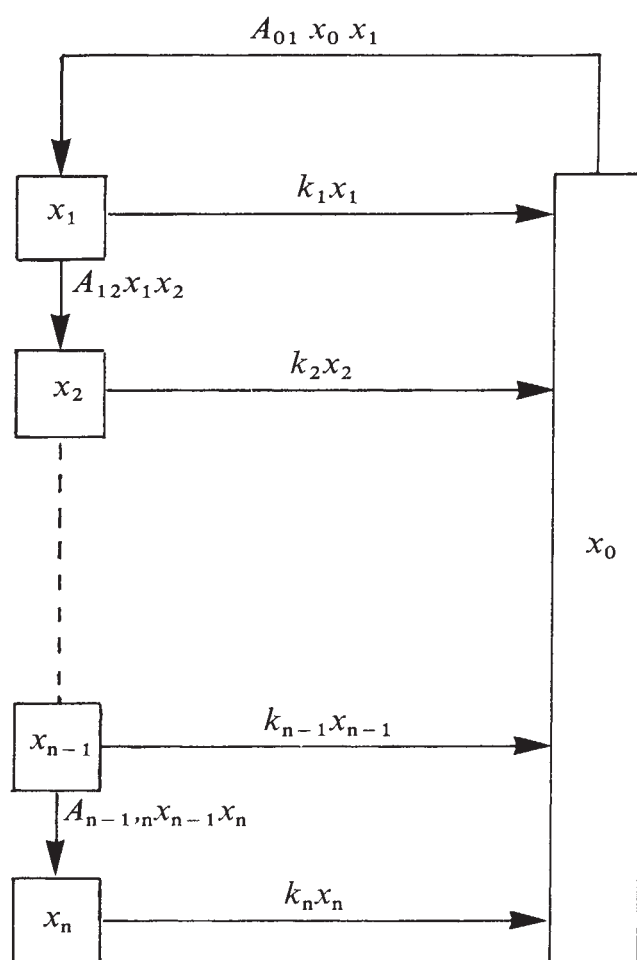


Fig. 1 Standing crops and mass fluxes in the proposed model of an n -level trophic chain.

fificantly influence the rate of provision of material for decomposition, and second, that decomposition is sufficiently fast for us to neglect the time delays in the decomposition process. This latter assumption seems reasonable in tropical and subtropical regions, but is dubious in boreal systems, where decomposition times are typically years (see ref. 6).

Provided only that the above system of equations possesses a steady state in which all of the standing crops are positive, the

system is always stable. We will now outline a proof of this result.

Routine but tedious neighbourhood stability analysis⁷ reveals that the system is stable if the $n \times n$ matrix B_n is stable (that is, if all its eigenvalues have negative real parts) where

$$B_n = \begin{bmatrix} -S_n & -S_n - t_{n-1} & -S_n & -S_n & \dots & \dots \\ S_{n-1} & 0 & -t_{n-2} & 0 & \dots & \dots \\ 0 & S_{n-2} & 0 & t_{n-3} & \dots & \dots \\ \vdots & \vdots & \vdots & \vdots & \ddots & \vdots \\ \vdots & \vdots & \vdots & \vdots & \vdots & \ddots \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \end{bmatrix} \quad (2)$$

$$\text{with } S_i = -A_{n-i+1, n-i} Q_{n-i+1}, \quad i = 1, 2, \dots, n$$

$$t_i = A_{n-i, n-i+1} Q_{n-i}, \quad i = 1, 2, \dots, (n-1) \quad (3)$$

and Q_i = Steady-state value of x_i

By a succession of column operations on the matrix $B_n - \lambda I$ (where I is the unit matrix) we find that

$$\det(B_n - \lambda I) = -S_n \det(B_{n-1} - \lambda I) + \det(A_n - \lambda I) \quad (4)$$

where A_n is the matrix obtained by setting $S_n = 0$ in the matrix B_n . The proof of stability now proceeds by induction. If the matrix B_{n-1} is stable we can use equation (4) to show that it is impossible for B_n to have any pure imaginary eigenvalues. Since the eigenvalues of a matrix depend continuously on the matrix elements, it follows that B_n is either always stable or always unstable. But for one particular choice of model parameters ($k_i = 1$ for all i) we can prove directly that B_n is stable by noting that if Q_i the steady-state value of x_i , is positive then the function

$$M(t) = \sum_{i=0}^n Q_i (x_i - Q_i)^2 \quad (5)$$

is a Liapunov function in some neighbourhood of the steady state. Thus if B_{n-1} is stable so is B_n . For $n = 1$ stability is obvious and so for any n the stability of B_n , and thus of the model, follows by induction.

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Anaerobiosis of fluid mud

OBSERVATIONS in estuaries have reported the existence of ephemeral deposits of semi-fluid mud^{1,2} extending several metres from the bottom. These muds differ from 'mud' as normally understood in that although they form definite boundaries with the overlaying water mass they have a lower solid content and settle, if at all, only very slowly. They are also reported to have only 4–400 mg ml⁻¹ suspended solids compared with an average of 1,300 mg ml⁻¹ for bottom muds³. Fluid muds can be detected only by high frequency (30 kHz) echo sounding techniques and have been found to vanish at certain periods of the tide cycle.

To obtain samples of fluid muds a special open-ended sampler⁴ was used, in conjunction with precautions to minimise microbial contamination. Samples were taken from masses located in the Bristol Channel by echo sounding with a Kelvin Hughes type MS26A echo sounder. The samples were returned to the laboratory for microscopic examination, estimation of bacterial numbers and measurement of the oxygen uptake.

Both wet and stained samples were examined by phase contrast and direct microscopy. Unlike ordinary mud from the Bristol Channel the samples contained little or no crystalline or unstainable material, and resembled a light floccular biological sludge. Organic carbon estimations showed between 2.5% and 7% organic carbon compared with 5% for Thames mud⁵ and ~15% for digested sewage sludge. Bacteriological counts were performed by the pour-plate method⁶ and duplicates incubated at 22 °C in the air, for 48 h and also anaerobically in a McIntosh and Fildes⁷ jar. Aerobic bacterial counts were 10⁷ per 100 ml and anaerobic counts 10³ per 100 ml.

Measurements on freshly collected liquid mud using a modified Clarke oxygen electrode⁷ showed the mud to be anaerobic immediately after collection and the rate of oxygen consumption to be 20 nmol ml⁻¹ min⁻¹. No samples of water from above the mud layer have shown dissolved oxygen values of <7 p.p.m. and most are close to saturation. It is reasonable to deduce, therefore, that the mud masses are anaerobic. That they have not been previously recognised as such probably arises from their characteristically 'aerobic' brown colour. It has been shown that proliferation of strictly anaerobic bacteria and the production of H₂S and black colours associated with metallic sulphides usually takes upwards of 5 d to develop subsequent to the onset of anaerobiosis⁸.

The high aerobic, and lower anaerobic counts suggest that these ephemeral muds may not persist long enough to become appreciably reduced before being dispersed in oxygenated waters at spring tides.

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To define true meaning

As any lexicographer knows, the meanings of words are not immediately open to introspection. Their covert nature underlies at least one current controversy: some theorists propose that meanings are composed of semantic primitives¹, whereas others deny the existence of such entities^{2,3}. We have investigated the matter in an evaluation of the responses of naive speakers as a source of lexicographical information.

According to a recent theory, there are two sorts of semantic primitive⁴. The first sort includes such notions as motion, vision, and possession, which are each the basis of a corresponding semantic "field". The second sort occurs across different semantic fields in a variety of semantic formulae; it includes such notions as action, causation, and intention. For example, the meaning of "x propels y" can be roughly analysed as "x does something that causes y to move", where "move" is the primitive that defines the field of motion, and "does something" and "causes" are ubiquitous primitives which occur in such similar formulae as "x does something that causes z to possess y", that is "x gives y to z", and "x does something that causes y to be visible to z", that is "x shows y to z".

The most elementary constituents of meaning are presumably various ineffable mental operations and states: these are the fundamental "particles" that make up the "atomic" primitives which seldom need to be analysed in the everyday use of language. It should consequently be relatively easy to produce a label for a semantic primitive but relatively difficult to define it. Therefore, the simpler a verb's semantic formula, the harder it should be to define the meaning of the verb: "atoms" should be harder to take apart than "molecules". For example, "move" should be difficult to define because it labels something that is virtually a primitive; "come" should be less difficult to define because its formula at least involves an adverbial modification, "move towards the location of speaker or some other specified location"; "bring" should be still easier to define because it involves the ubiquitous primitive of causation, "cause to come with"; and "chase" should be the easiest of these verbs to define because it introduces the further ubiquitous primitive of intention, "move rapidly after as a result of an intention to catch". We predicted that the order of difficulty from easiest to hardest for the four corresponding generic classes of verbs would be: intentional verbs, causative verbs, adverbially modified verbs, and primitive verbs.

In a preliminary study to test this prediction, eight undergraduate students each attempted to define sixteen words (verbs of the four classes drawn from each of the semantic fields of motion, vision, possession and communication). We tried to choose words with the same frequency of usage, but there was a slight tendency for the intentional verbs to be less commonly used. Half the subjects received one set of sixteen words, and the other half received another set of sixteen words. Each word was presented separately with a sentence exemplifying the particular sense to be defined. The subjects, who were tested individually, were told to imagine that they were defining the words for the benefit of a child or a foreigner with an imperfect grasp of the language. They wrote down their definitions in their own words and in their own time. After they had finished this task, they put the verbs into a rank order corresponding to their subjective impression of the difficulty of defining them.

Something of the flavour of the subjects' definitions is conveyed by the following examples: chase: (1) to follow closely—indication of speed and urgency; (2) he

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⁷ Chappell, J. B., *Biochem. J.*, 90, 225–237 (1964).

followed the dog and tried to catch it; (3) to chase is sometimes to follow it with the intention of catching up with it; (4) to pursue in order to catch or overtake. Example (2) is one of the few cases where a subject has ignored the instructions and couched the definition of a word in terms of the sentence illustrating its meaning. Despite the superficial variety of the definitions, an amalgam of them captures the essential meaning of the word. In fact, example (4), which is presented for purposes of comparison, was culled from a recent dictionary compiled with considerable linguistic sophistication¹. As anthropologists have known for some time, naive informants can on occasion yield accurate and relevant lexicographical information.

Some of the definitions consisted of synonyms or words with more specific or recondite senses than the *definiendum*. They could hardly be helpful to children or foreigners. Such definitions were particularly prevalent for primitive verbs, accounting for just under 70% of the responses to them. The prevalence of synonyms in the definition of this class of verb attests to the difficulty experienced in attempting to explain their meaning. In addition, it makes it difficult to include this class when rank ordering the accuracy of each subject's definitions. In contrast, it was a relatively straightforward matter to rank order the accuracy of each subject's definitions for the three remaining classes of verb within a given semantic field. Thus, granted our earlier analyses of "come," "bring" and "chase," the following definitions from one subject are ranked in order of increasing accuracy: (1) "come": "to come to a place or situation means to change the place or situation from a different place or situation to the first mentioned place or situation"; (2) "bring": "when something moves from some place to another and takes something with it"; (3) "chase": "to chase is sometimes to follow it with the intention of catching up with it". The first definition fails to mention either movement or the crucial role of the speaker's location; the second definition mentions movement but is not as helpful as it could be about the causal relation since it resorts to a near synonym, "take"; the third definition is very nearly complete. The mean ranks of the accuracy scores were as follows: adverbially modified verbs, 2.4; causative verbs, 2.8; intentional verbs, 3.1; and the ranks conformed in a highly reliable manner to the predicted trend (Page's $L=106$, $P=0.01$). The subjects' own impressions of the difficulty of the task, as revealed by their rank orders of the verbs, also conformed reliably to the predicted trend. Their mean ranks were as follows: intentional verbs, 5.3; causative verbs, 8.4; adverbially modified verbs, 9.5; and primitive verbs, 10.7 (Page's $L=225.5$, $P < 0.01$). The numbers of words and clauses in a definition were not related to its accuracy or subjective difficulty. However, subjective difficulty was negatively correlated with the accuracy score in a highly reliable way (Page's $L=4375.5$, $P < 0.005$).

The predicted order of difficulty was confirmed in two further studies both of which used the measure of accuracy employed in the original experiment. The first study examined 5 subjects' spontaneously spoken, rather than written, definitions: their accuracy was in accord with the predictions. The second study, carried out by Marla Petal, again replicated the trend using the written definitions of both Japanese and English words. Three English-Japanese bilinguals and four English-speaking mono-linguals served as subjects in this experiment.

Of the twenty subjects so far tested, 11 have an overall trend that conforms exactly to the prediction, 7 have an overall trend more in accord with the prediction than against it, and only two subjects have produced results definitely incompatible with the prediction ($P=0.0003$, Sign

test). We conclude that some common words are, indeed, easier to define than others.

Words that can be adequately captured in a definition ought accordingly to have formalised senses, that is, they should be less likely to take on new meanings than words that are less adequately defined. Consequently, the number of different meanings of the 32 words used in our original study would be expected to increase as their semantic formulae became more simple. We tested this prediction by recourse to the dictionary². The mean frequencies of separate numbered meanings in the dictionary³ of the 32 words used in our first study were as follows: intentional verbs, 5.4; causal verbs, 8.8; adverbially modified verbs, 10.3; and primitive verbs, 14.8 (a trend that just fails to reach significance, Page's $L=212.5$, $P>0.05$). This phenomenon and the results of the experimental studies can be explained by postulating the existence of semantic primitives that are easy to label but hard to define. We do not wish to imply that primitives are necessarily retrieved in every linguistic task—a listener may well understand a sentence without having to break it down into atoms. However, it seems that primitives are verbalised in a task that requires naive informants to act as lexicographers and to define the true meaning of words.

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Tidal rhythm in a seabird

GUILLEMOTS (*Uria aalge*) breed on cliff ledges in densely packed colonies often numbering many thousands of pairs. About 25% of the British population of these birds nests in Orkney¹, where their proximity to North Sea oil developments makes it important that the numbers breeding are continuously monitored to check for any adverse effects. One of the difficulties in estimating the breeding population is that the numbers present on cliff ledges show considerable variation, particularly before egg-laying². The data presented here were collected on the island of Copinsay, Orkney, between April 20 and May 13, 1976 with a view to determining the reasons for these fluctuations. They demonstrate the clear effect of a tidal rhythm, a phenomenon previously described for birds which feed close inshore^{3,4}, but not apparently for any species which feeds in deeper water, though Tuck⁵ makes passing reference to the possibility of such an effect at some of the guillemot colonies which he studied in Canada.

Data were collected at a study colony of roughly 80 pairs. The number of birds present on the cliff was counted at 0800, 1200, 1600 and 2000 BST on each day. Figure 1 shows the results of these counts over a 16-d period and indicates substantial variance both within days and between counts at the same time on different days. There was, however, no significant tendency for the counts on some days to be higher than those on others ($0.10 > P > 0.05$, Friedman two-way analysis of variance). As shown in Table

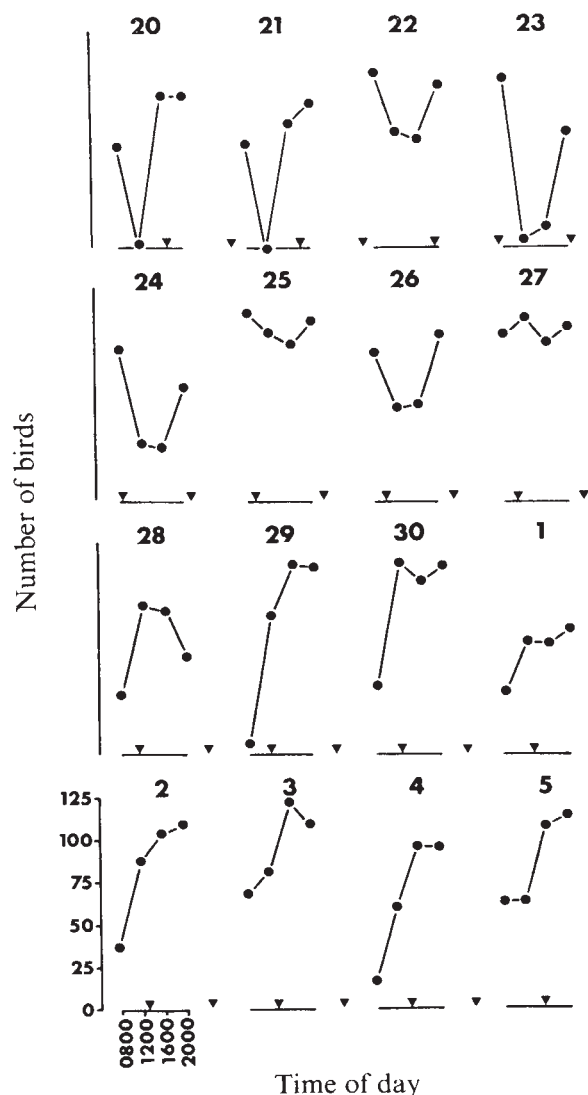


Fig. 1 Number of guillemots present at a study colony at four different times of day on 16 successive days from April 20 to May 5, 1976. Arrows indicate times of high tides.

1, two main factors were found to contribute to the variance between counts: a tendency for numbers to increase during the day and a tendency for them to be highest at around high tide. This tidal effect can be seen in Fig. 1. Initially, the main rise in numbers during the day is between 1200 and 1600 h; on subsequent days it becomes progressively later until by May 5 it occurs in the same interval once again. A 16-d period was chosen for analysis because high tide shows a similar shift round the 24 h, being at 1649 on April 20 and 1655 on May 6. The

Table 1 Variations in numbers of guillemots present with time of day and with state of tide

Time of day (BST)	0800	1200	1600	2000	
Mean number present (N)	63.1 (16)	63.6 (16)	81.8 (16)	93.8 (16)	$P < 0.05^*$
Time since high tide (h)	0-3½	3½-6½	6½-9½	9½-13	
Mean number present (N)	91.9 (16)	75.5 (17)	56.1 (19)	84.8 (12)	$P < 0.05^\dagger$

*Friedman two-way analysis of variance.

†Kruskal-Wallis one-way analysis of variance.

data are not therefore biased towards one state of the tide rather than another.

Observations were also made on the number of birds arriving at and departing from the cliff during 36 1-h watches carried out immediately after some of the counts. Of these, 13 watches were discarded because they were preceded or interrupted by disturbance from gulls calling: at this stage of the season this led to departure of many guillemots from the cliff and their gradual return during the subsequent half-hour. Movements during the remaining 23 watches were analysed to examine the influence of time of day; the same watches were combined with six at other times of day to see whether arrivals and departures were influenced by the state of the tide (Table 2). There was no significant difference in the number of birds arriving at the cliff between the four times of day but departures did vary, being greatest during watches starting at 2000. By contrast, departures did not vary with state of tide while arrivals showed significant variation, the greatest numbers returning to the cliff when the tide was flowing.

Table 2 Variations in arrivals and departures of guillemots with time of day and with state of tide (1-h watches).

Time of day (BST)	0800	1200	1600	2000	
Mean arrivals	21.8	18.0	26.4	26.4	n.s.*
Mean departures (N)	16.6 (8)	16.4 (5)	18.2 (5)	47.0 (5)	$P < 0.02^*$
Time since high tide (h)	0-3½	3½-6½	6½-9½	9½-13	
Mean arrivals	14.0	14.8	27.9	29.3	$P < 0.02^*$
Mean departures (N)	14.7 (6)	14.0 (4)	20.3 (11)	33.0 (8)	n.s.*

*Kruskal-Wallis one-way analysis of variance.

These results indicate a strong influence of state of tide on the number of guillemots present on breeding ledges before egg-laying. The most likely reason for this is that food availability varies with the tide, being greatest at or shortly after low tide, so that birds returning to the cliff after feeding do so mainly when the tide is flowing. Observations of birds carrying fish suggest that on Copinsay, as on the Farne islands⁶, guillemots feed mainly on sandeels (*Ammodytes* spp.), which they catch in mid-water some distance off-shore⁷. The pattern of tidal flow round Orkney is complex⁸, with many areas of turbulence at points where two tidal streams meet. Such disturbance, which varies with the tidal cycle, may bring more sandeels towards the surface and thus within the range of a diving guillemot. Strong currents, such as that which flows eastwards through the nearby Pentland Firth when the tide on Copinsay is rising, may also make food more available by moving shoals of fish through the feeding area. No effect of tides could be found when the observations were repeated in June, at a time when sandeels are abundant and fluctuations in their availability are unlikely to be marked.

In addition to the effects of time of day and of state of tide, the weather also affected the number of guillemots present. A negative correlation was found between wind speed and the average daily count during April/May (Spearman rank correlation coefficient, $r_s = -0.458$, $n = 24$, $P < 0.05$). There was too little rainfall in this period for its effect to be assessed, but during June there was a significant tendency for the second of two counts to be lower when rain occurred during the intervening 4 h (Mann-Whitney U test, $P = 0.004$).

Clearly the number of guillemots present on cliff ledges is affected by a wide range of environmental factors and monitoring procedures must be devised to take account of this.

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H-Y antigen and the origin of XY female wood lemmings (*Myopus schisticolor*)

THE wood lemming, *Myopus schisticolor* Liljeborg, is distinguished by an aberrant sex ratio, with a considerable excess of females, and by the fact that some females produce only daughters^{1,2}. Fredga and his associates³ have provided a basis for understanding both these characteristics. They observed that 82 out of 181 female wood lemmings studied (45%) had an XY sex chromosome constitution, with chromosomal G-banding and C-banding patterns indistinguishable from those of XY males. On the other hand, the XY females were anatomically normal and indistinguishable from XX females. Furthermore, meiotic studies³ showed that the germ line in the somatically XY females was XX. The second X chromosome in the germ cells must have arisen by non-disjunction from the single X present in the XY cells. Thus all progeny from such females must have received copies of the same X chromosome. How does one reconcile these observations with the apparent function of the Y chromosome in mammalian sex determination⁴, or with more recent evidence that a particular Y-linked gene which controls presence of H-Y antigen is critical for differentiation of the male gonad? We have approached these questions serologically by typing male and female wood lemmings for expression of H-Y

antigen, and we have found that all female wood lemmings are H-Y⁻.

H-Y antigen is a cell surface component that is present in males of all mammalian species tested, including the human⁵. In intersexual or sex-reversed individuals, testicular differentiation is more closely correlated with presence of H-Y antigen than it is with presence of an intact Y chromosome⁶. Intersexual X(*Tfm*)/Y mice⁷ and their human counterpart, XY individuals with testicular feminisation syndrome⁸, are H-Y⁺. So are *Sxr*/XX (sex reversed) male mice⁹, as well as human XX males and XX true hermaphrodites⁹. It was therefore critical to ascertain whether lack of testicular differentiation in the somatically XY female wood lemming is associated with absence of H-Y antigen. For this study, 12 animals from the colony at Lubeck were shipped to the Memorial Sloan-Kettering Cancer Center. Multiple tissues (brain, liver, and spleen) were taken for H-Y antigen determination, using procedures fully described elsewhere^{6,12}. Lung and heart were cultured for chromosome studies, according to Miller *et al.*¹⁰.

The four males were H-Y⁺ and the eight females were H-Y⁻ (Table 1). Two of the females had XY karyotypes indistinguishable from those seen in the two successfully karyotyped males. One of the females was an XY/XX mosaic with almost equal proportions of the two cell types scored in cultured lung fibroblastic cells (Fig. 1). Thus in the wood lemming, males are H-Y⁺ and females are H-Y⁻ irrespective of karyotype. The absence of H-Y antigen in XY or XY/XX mosaic females may be responsible for the absence of testes in these females because H-Y antigen appears to act as a 'cell recognition signal' that is essential for virilisation of the indifferent embryonic gonad^{6,11,12}. But what is responsible for the absence of H-Y antigen in these animals, whose Y chromosome looks perfectly normal?

A mutation at the H-Y locus on the Y chromosome might render the gene product both non-antigenic and incapable of performing its essential function. The original mutation would arise in the male germ line, and thereafter XY females could only arise from XY females. But since the germ line in all XY female wood lemmings tested was XX, the Y chromosome in these females could not be transmitted³, and a mutation at the H-Y locus is thus ruled out.

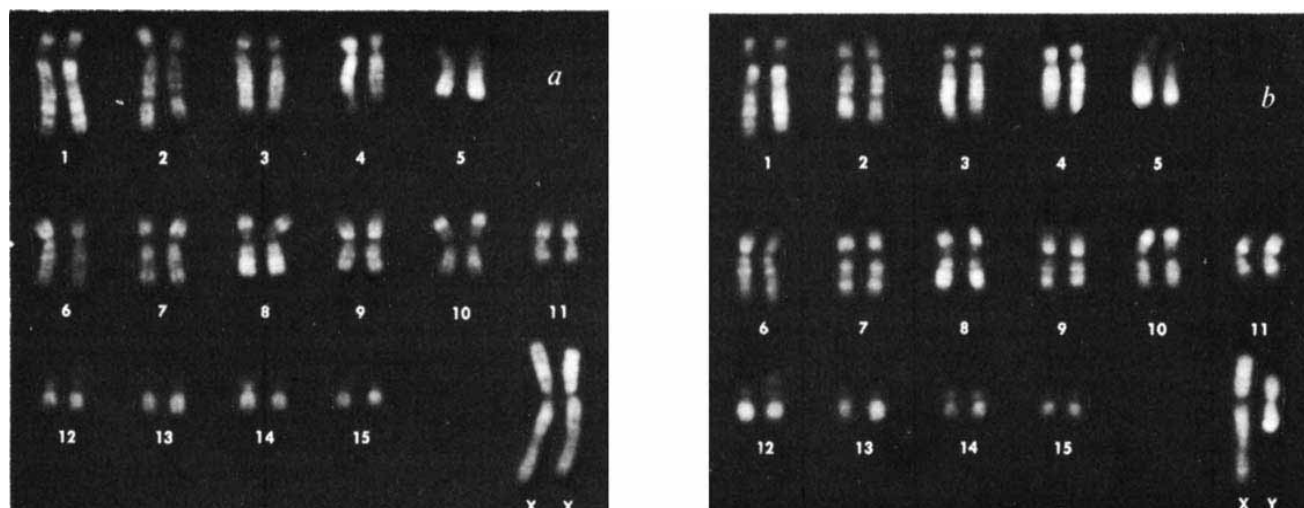


Fig. 1 Quinacrine fluorescence karyotype of *a*, an XX cell and *b*, an XY cell from an H-Y antigen negative female wood lemming.

The most likely explanation for the H-Y⁻ phenotype of XY female wood lemmings is that there is an X-linked 'sex reversal' mutation that blocks the expression of H-Y antigen. XY females could arise from matings involving either XX or XY females according to this scheme, which is consistent with the unpublished observations of Frank, Winking and Fredga. Furthermore, loss of the Y chromosome, and non-disjunction of the X to produce an XX germ line in somatically XY females³ would provide a

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Table 1 Sex, karyotype and H-Y antigenic status

Animal no.	Sex	Karyotype	H-Y Antigen
1645	♂	XY	+
1997	♂	XY	+
1567	♂	?	+
1798	♂	?	+
2047	♀	XY	-
2308	♀	XY	-
2088	♀	XY/XX	-
2140	♀	?	-
2259	♀	?	-
2397	♀	?	-
2407	♀	?	-
2258	♀	XX	-

Tests for H-Y antigen were performed according to procedures fully described elsewhere^{6,12}. Briefly, mouse H-Y antisera were selected, pooled, and the pools divided into three parts. One part was unabsorbed and the other two parts were absorbed with cells from female and male wood lemmings, respectively. Positive absorption (indicating presence of H-Y antigen on the absorbing cells) was manifested as a decrease in reactivity of absorbed sera (decrease in number of mouse sperm killed in the cytotoxicity test and fall in the number of mouse sperm labelled in the mixed haemadsorption-hybrid antibody test; see ref. 12).

basis for obtaining all-female litters, since all the XY progeny would receive an X chromosome with the mutant gene. Our finding that one female was an XY/XX mosaic and the not infrequent occurrence of other sex chromosomal anomalies (XO, XYY, XXY) in the laboratory bred stock of *Myopus* suggest that the mechanism by which the Y chromosome is lost and the X chromosome constitution doubled may represent a more general phenomenon not always restricted to the germ line. And this raises the question of what triggers these events and the range of variability in its timing. For example, could some XY females have XY germ cells? If so, then XY females could occasionally produce male progeny even though they usually do not.

Whether XY wood lemmings develop as males or females seems to depend on whether or not H-Y antigen is expressed on their cells, and this in turn depends upon an X-linked gene as well as the Y-linked H-Y locus itself. In view of the evolutionary conservation of the mammalian X chromosome⁴, a similar X-linked gene may be present in other mammals as well. Indeed the familial occurrence of XY females with gonadal dysgenesis in the human species may be due to a mutation at the same gene locus. Because they have streak gonads and are therefore sterile, affected individuals can arise only from matings involving normal XX females.

The pattern of inheritance is that of an X-linked recessive or autosomal dominant but sex-limited trait, with a single copy of the mutant gene sufficient to suppress testicular differentiation¹³⁻¹⁵. By analogy with the wood lemming, and in view of the critical role of H-Y antigen in directing testicular differentiation, we would expect the XY females in these families to be H-Y⁻. On the other hand, sporadic cases could arise as a result of a spontaneous mutation involving either the same X-linked locus or the Y-linked H-Y determinant.

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T-cell idiotypes are linked to immunoglobulin heavy chain genes

FOUR gene clusters have products that seem to participate in antigen binding on T or B lymphocytes. Three (the genes coding for the heavy and light chains of immunoglobulin molecules) are known to code for structural elements of B-cell receptors for antigen. There is evidence that a fourth group (the immune response genes associated with the major histocompatibility complex (MHC) locus) generates T-cell recognition structures for

Table 1 No linkage between inheritance of T-cell idiotypes and major histocompatibility locus genes

Animal no.	Sex	Phenotype	Uptake of anti- (Lewis anti-DA) anti-idiotype (mean c.p.m. $\pm$ s.e. of triplicates)	
1	male	L $\times$ BN	+	6,313 $\pm$ 168
2	male	L $\times$ BN	—	3,394 $\pm$ 85
3	male	BN	—	2,918 $\pm$ 360
4	male	L $\times$ BN	+	8,216 $\pm$ 253
5	male	L $\times$ BN	+	5,668 $\pm$ 271
6	male	BN	—	2,905 $\pm$ 218
7	male	BN	+	6,251 $\pm$ 97
8	male	L $\times$ BN	—	3,632 $\pm$ 73
9	male	BN	+	6,267 $\pm$ 678
10	male	L $\times$ BN	—	3,643 $\pm$ 212
11	male	BN	—	3,508 $\pm$ 118
12	female	BN	—	3,163 $\pm$ 148
13	female	BN	—	2,950 $\pm$ 74
14	female	L $\times$ BN	+	6,050 $\pm$ 162
15	female	BN	+	5,949 $\pm$ 216
16	female	L $\times$ BN	—	3,183 $\pm$ 319
17	female	L $\times$ BN	+	6,247 $\pm$ 59
18	female	L $\times$ BN	+	5,911 $\pm$ 416
19	female	L $\times$ BN	—	3,175 $\pm$ 43
20	female	BN	—	2,859 $\pm$ 59
21	female	L $\times$ BN	—	3,459 $\pm$ 338
22	female	L $\times$ BN	—	3,490 $\pm$ 179
23	female	BN	+	8,049 $\pm$ 856
Controls				
Lewis			+	9,361 $\pm$ 905
BN			—	3,151 $\pm$ 51

* + Denotes specific uptake, — denotes no specific uptake.

Individual offspring from mating (L $\times$ BN) F_1 hybrids with BN rats were typed for Ag-B phenotype using Lewis anti-BN and BN anti-Lewis alloantisera and ^{125}I -labelled protein A as a marker². For determination of anti-idiotype uptake 1×10^7 purified T lymphocytes were incubated with anti-(Lewis anti-DA) antiserum followed by ^{125}I -labelled protein A as a marker².

antigen¹. No evidence exists that any of these four gene groups are linked. In a study of the T-cell receptors for antigen we used anti-idiotypic antibodies against specific T-cell receptors²: T and B cells specific for the same antigen were shown to express similar idiotypes². Analogous findings using other systems and approaches have been reported^{3,4}. Together these results strongly suggest that antigen-binding receptors on T and B lymphocytes at least in part make use of the same genetic material. Accordingly, T-cell receptors should at least partly be coded for by genes linked to "conventional" immunoglobulin gene groups. We prove here that idiotypic T-cell receptors with specificity for MHC antigens in the rat are coded for by genes linked to the heavy chain immunoglobulin genes.

Our experimental setup consists of inbred strains of rats (Lewis, BN or DA) which differ as to the rat MHC genes, the Ag-B or H-1 locus. Lewis and DA also differ with regard to allotypes of the IgA class and of the κ light chains⁵⁻⁷. Lewis T cells with immune

reactivity against, for example, Da MHC antigens carry distinct idiotypes different from those on Lewis T cells with reactivity against BN antigens². Anti-idiotypic antisera against the Lewis T receptors with specificity for the DA Ag-B antigens were produced by immunisation of (Lewis $\times$ DA) F_1 rats with Lewis T lymphocytes². Anti-allotype sera specific for the Lewis IgA or κ light chain allotypes were prepared as previously described⁵. Using this setup we started to investigate whether the Lewis anti-DA T-cell idiotypes were inherited in a manner indicating linkage to either the MHC locus or the heavy chain or the κ light chain gene clusters.

A direct search for linkage between T-cell idiotypes and MHC locus genes was first carried out. Using the described anti-idiotypic antiserum we analysed the T lymphocytes of individual offspring from mating of (Lewis $\times$ BN) F_1 hybrids with BN rats. Such rats were typed for their respective Ag-B composition and presence or absence of the Lewis anti-DA idiotypes on some of their T lymphocytes. Table 1 shows that the T-cell idiotypes are not linked

Table 2 Linkage between inheritance of T-cell idiotype and heavy chain allotypes (uptake of anti-idiotype)

F_2 rat no.	Lewis heavy chain	Lewis light chain	Uptake of radioactivity (mean c.p.m. $\pm$ s.e. of triplicates)	
			Incubated with anti-(Lewis anti-DA) anti-idiotype	Incubated with (Lewis $\times$ DA) F_1 normal serum ¹
1	—	—	3,813 $\pm$ 113	3,787 $\pm$ 73
2	—	+	4,106 $\pm$ 254	3,925 $\pm$ 48
3	—	+	4,478 $\pm$ 57	3,783 $\pm$ 166
4	+	+	5,816 $\pm$ 344*	4,016 $\pm$ 63
5	+	+	5,675 $\pm$ 146*	3,872 $\pm$ 41
6	+	+	5,824 $\pm$ 34*	3,890 $\pm$ 104
7	+	+	7,218 $\pm$ 386*	3,933 $\pm$ 109
8	+	+	7,395 $\pm$ 199*	3,824 $\pm$ 34
Controls				
Lewis				
1	+	+	8,234 $\pm$ 319*	3,960 $\pm$ 74
2	+	+	8,432 $\pm$ 98*	3,801 $\pm$ 37

* Specific uptake of anti-idiotype antibodies.

(Lewis $\times$ DA) F_2 rats were typed for Ag-B antigens using Lewis anti-DA and DA anti-Lewis alloantisera and ^{125}I -labelled protein A as a marker². Only those rats homozygous for the Lewis Ag-B antigens were used. Sera of such rats were tested for presence or absence of the respective allotypes using gel diffusion technique. For determination of anti-idiotype uptake see Table 1.

to the MHC genes. Roughly half of the rats typing as homozygous for the BN Ag-B and half of those typing as (Lewis × BN) F₁ hybrids were positive in the radioimmunoassay detecting the idiotypic.

In a second series of experiments we looked for linkage between T-cell idiotypes and heavy or κ light chain allotypes. (Lewis × DA) F₂ rats were typed for Ag-B antigens and only those homozygous for the Lewis Ag-B antigens were used for the subsequent tests. Sera from such rats were obtained and tested individually for presence or absence of the respective allotypes using gel diffusion techniques. The rats were then killed and their T lymphocytes from spleen and lymph nodes obtained after filtration through anti-Ig columns². Samples of the T cells were used in a radioimmunoassay with the anti-idiotypic serum to determine whether or not the cells contained the Lewis anti-DA idiotypes². The remaining cells were used in MLC cultures against DA or BN stimulator cells after the responder cells had been treated with anti-idiotypic or normal serum in the presence of complement². Table 2 shows that three out of eight F₂ rats typed as being homozygous for the DA heavy chain genes (they did not express the Lewis IgA allotype in their

T cells by physical and functional criteria in these animals with the appropriate heavy chain allotype, taken together with the findings in the mouse using an entirely different antigenic system, would seem to solidly establish this inheritance pattern of T-cell idiotypes.

Is it possible to reconcile the present findings with the reported antigen-specific T-cell-derived molecules carrying serological markers indicating them to be products of the IR genes^{9,10} mentioned earlier? Could they, for instance, be the very same molecules where one chain carried the idiotypes detected in the present assays, and another chain carried the Ia-associated antigenic markers? Our analyses of the biochemistry of the idiotype T-cell receptors have, unfortunately, so far failed to indicate that this is true. When isolated from T-lymphocyte supernatants or serum our T-cell-derived, antigen-binding and idiotype molecules have a molecular weight of ~150,000 (ref. 11). These molecules can be split into two single polypeptide chains still expressing idiotypes and antigen-binding ability with a molecular weight of slightly above 70,000. These single chains can then be further degraded into molecules of a size range from 30,000

Table 3 Linkage between inheritance of T-cell idiotypic and heavy chain allotypes (MLC inhibition)

F ₂ rat no.	Lewis heavy chain	Lewis light chain	MLC inhibition* against DA stimulator cells	MLC inhibition* against BN stimulator cells
1	—	—	0	0
2	—	+	15	0
3	—	+	12	13
4	+	+	71	0
5	+	+	87	0
6	+	+	83	3
7	+	+	74	0
8	+	+	90	10
Controls				
Lewis				
1	+	+	94	0
2	+	+	95	1

*Responder lymphocytes from F₂ rats or normal Lewis control rats were incubated with anti-Lewis anti-DA/anti-idiotypic antiserum and complement before initiation of the MLC². Figures denote % reduction compared to the control treated with (Lewis × DA)F₁ normal serum and complement. For further details see text of Table 2.

sera). Precisely these three rats were also negative with regard to the expression of the Lewis anti-DA T-cell idiotypes, whereas all the other five rats were positive. This thus strongly indicated that the T-cell idiotypic-coding genes are linked to the genes coding for the heavy chains of conventional immunoglobulins.

The inheritance of the Lewis κ light chain allotype did not show a similar pattern *vis-à-vis* the T-cell idiotypes. One of the eight rats typed as homozygous for the DA κ light chain genes and this rat also happened to be in the idiotypic negative group. Two rats, however, were idiotypic negative but Lewis κ light chain allotype positive.

Final proof that the radioimmunoassay did indeed detect the inheritance patterns of functionally relevant T-cell receptors came from the experiments on the inhibition of MLC using anti-idiotypic antisera (see Table 3). Treatment of the individual Lewis spleen cell suspensions with anti-(Lewis anti-DA) idiotypic serum plus complement never caused any reduction in MLC reactivity against BN MHC antigens. However, T cells from five suspensions displayed highly significant reduced activity against DA antigens after such treatment, whereas three suspensions retained normal reactivity against DA. The five with reduced activity were derived from the five F₂ rats which were positive for Lewis heavy chain allotype, whereas the "reduced" suspensions came from the three rats typing as homozygous for the DA heavy chain immunoglobulin genes. Chi-square analysis of data demonstrated the correlations between idiotypic and allotype to be highly significant. It could thus be conclusively shown by two aspects that the inheritance of T-cell idiotypes is linked to heavy-chain allotype expression.

A similar conclusion has been reached using heterologous anti-idiotypic antibodies in the mouse to activate specific helper cells⁸. The present direct demonstration of the inheritance of idiotypes on

to 40,000 (refs 11, 12). Only the latter molecules have sizes resembling those reported for the Ia-associated T-cell factors and our 30,000–40,000-dalton molecules can be shown to be single chains with idiotype determinants (the constitution as to chain type of the Ia-positive antigen-specific T-cell molecules is unknown but Ia antigens in general are two-chain structures¹³). Furthermore, we have so far been unable to detect any additional chain, nor have we found serological markers of Ia type on our idiotype-positive T-cell molecules. Molecules similar to ours in size and serology have also been isolated from mouse T cells¹⁴ adding further weight to our statements. It would thus seem clear that no simple model exists as yet that can reconcile the reports on specific factors from T cells being coded for by Ir genes linked to MHC genes with the present ones showing linkage of T-cell idiotypic expression to heavy chain immunoglobulin genes.

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Antibody patterns in genetically identical frogs

THE origin of antibody diversity is still unclear. Either the 10^4 to 10^8 different antibody specificities of the vertebrate immune system are completely encoded in germ line genes, or somatic mutations or recombination of a few genes contribute substantially to diversity. Both the large pool of specificities for single antigens and the very heterogeneous specificities among different individuals of inbred mouse strains (which are assumed to be genetically homogeneous) have led to somatic mutation theories. We have studied the immune response in *Xenopus* frogs which can be heterozygous and at the same time genetically identical. If there are many identical antibodies against several antigens in different individuals, somatic changes of genes coding for antibody specificities cannot be important for antibody variability in such animals. Antibodies to two different antigens have been assayed for by two techniques: inactivation of antigen-coupled phages by antibodies including inhibition of inactivation by free antigen; and isoelectric focusing (IEF). The results show low variability of antibody specificity, and often identical patterns among different individuals having identical genetic background.

Crosses of *X. laevis* (L) with *X. gilli* (G) yield female (LG) offspring which lay diploid eggs, the genome of which is

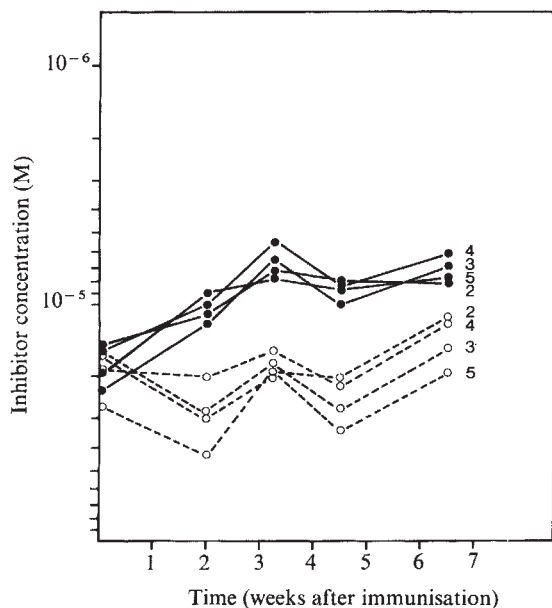


Fig. 1 Inhibition with DNP-lysine (●), and TNP-lysine (○) of the inactivation of DNP-T₄ phage by serum of isogenetic *Xenopus* hybrids immunised with 20 µg DNP-KLH. Difference in concentration of ligand necessary for 50% inhibition as a function of time after injection. Each number corresponds to a single animal.

identical to that of the mothers. The gynogenetic development (that from the female pronucleus alone) of these eggs gives rise to individuals which are genetically identical; that is, they form a clone¹. After a single injection of 20 µg dinitrophenyl-Keyhole limpet haemocyanin (DNP-KLH) in Freund's complete adjuvant into these isogenetic animals, the level and relative affinities of anti-DNP antibodies were measured by the technique of inactivation of DNP derivatised bacteriophage T₄. The kinetics of the response (that is the inactivation constant of the serum as a function of

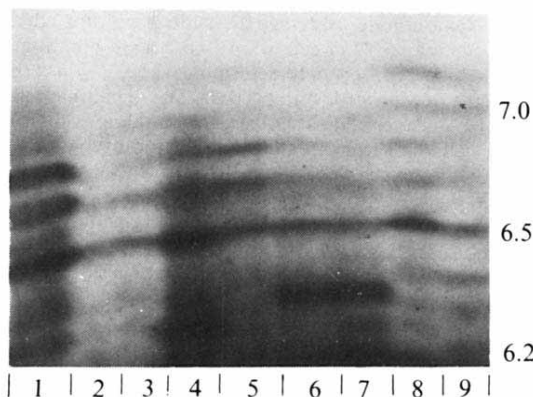


Fig. 2 Anti-DNP sera of 9 individuals of clone LG₁₇, secondary response. The animals were first injected with 20 µg DNP-KLH, and 36 weeks later with 40 µg DNP-KLH. Sera were collected 3 weeks after the reinjection. Individuals 1-5 show identical IEF spectrotypes. Individuals 6-9 which show different clones in addition to a common LG₁₇ spectrotypes had previously rejected a skin graft of *X. laevis*. The primary anti-DNP response after 10 weeks, though weaker, was almost identical to the secondary response in this case.

time) were superimposable for each isogenetic animal from a given clone, whereas for outbred *X. laevis*, the responses differed². The same was true for their discriminatory capacity between DNP and TNP (trinitrophenyl-) groups over a seven-week period, as measured by inhibition of phage inactivation (Fig. 1).

Antibodies titrated by phage inactivation are predominantly IgM³. These antibodies are the first to appear in this response, and they were always present during it.

Three to six weeks after injection of antigen, low molecular weight antibodies to DNP appeared. The patterns of these antibodies as evaluated by IEF^{4,5} revealed restricted heterogeneity and almost identical patterns for different individuals (Fig. 2). Of the five clones studied, three had the same antibody patterns (LG₇, LG₁₅, LG₁₇). These three

Fig. 3 Anti-SRBC sera of individuals of two clones, collected 6 weeks after immunisation with a single dose of 10⁹ SRBC. After IEF the gels were overlaid with SRBC in 0.5% Agarose containing guinea pig complement. Individuals 1-4 belong to clone LG₁₅, from 5-7 to clone LG 14. No. 8 is a prebleed.

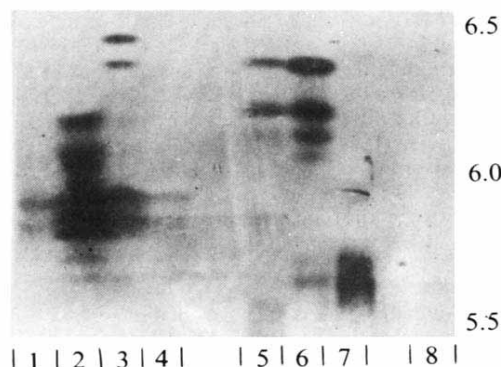
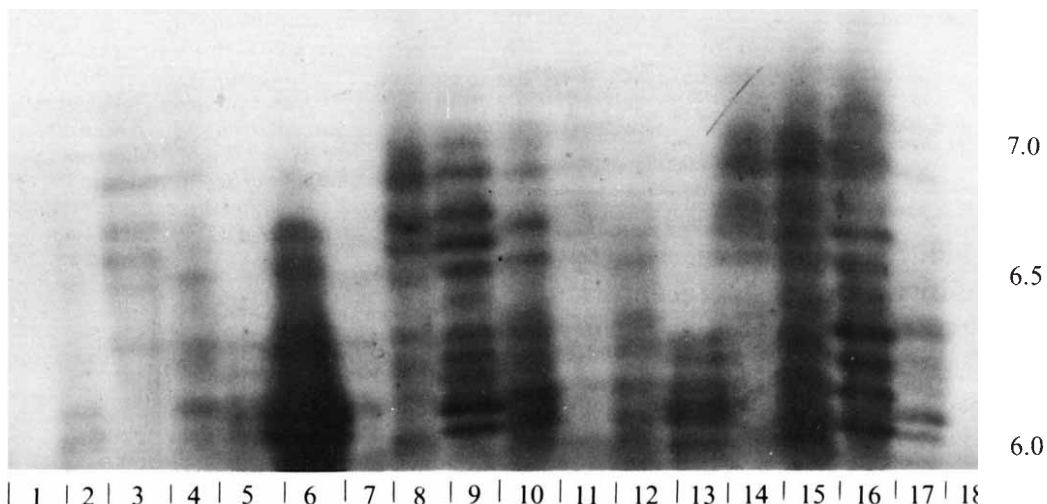


Fig. 4 Anti-DNP sera of 17 outbred individuals of *X. laevis*. The animals were injected with 40 µg DNP-KLH, and 13 weeks later with 40 µg DNP-KLH. Sera were collected 2 weeks after the second injection. Animal 18 was injected with streptococcal carbohydrate.



clones are identical at the major histocompatibility complex (MHC), as determined by the mixed lymphocyte reaction⁶. A fourth clone, LG₅, shares one MHC haplotype with the other three. Some of its members also displayed this IEF pattern of LG₁₅, LG₁₇ and LG₇; other members exhibited different patterns. The two members of a fifth clone (LG₂) tested displayed an identical antibody pattern which was different from that of the other clones. These animals also differed from those in the other clones by at least one MHC haplotype. Some individuals in a clone had rejected a skin graft from a genetically unrelated *X. laevis* donor before they were immunised with DNP-KLH. These individuals partially differed in their antibody patterns (see animal 6 and 7, Fig. 2, and less prominently 8 and 9). Thus, 'environmental' action on the immune system may influence the immune response pattern as evaluated by IEF.

Hapten-binding proteins were detected in one series of experiments by radioactive hapten binding and subsequent autoradiography⁴. In a second series antibodies were identified by complement-dependent lysis of TNP-derived (SRBC)⁵. With this technique, the same results as with radioactive hapten binding were obtained. The immunoglobulin nature of these proteins was demonstrated by the following observations: First, no antibody bands were seen in the pre-immune serum. Second, a pool of sera from animals of one clone was passed over an anti-immunoglobulin adsorbent column and subsequently did not show the bands. When the pooled sera were passed over an anti-µ column, however, the hapten-binding pattern on the IEF gel was present. Third, in the case of the TNP-SRBC detection system, no lysis could be seen without complement.

Given the assumption that identical banding in IEF gels reveals identity in primary antibody structure, the primary anti-DNP response in *Xenopus* seems to be due to the expression of germ line V genes. If somatic diversification had occurred, it occurred non-randomly, or rarely.

Obviously, the structural genes present in the germ line can code for at least some functional antibodies, regardless of any other mechanism for further diversification. Nevertheless, if somatic events are, in fact, necessary for generating antibody diversity, a substantial number of antigen specificities should not be covered by germ line genes. Thus we studied the immune response in the isogenetics to another antigen that displays many different antigenic determinants. The animals received a single dose of 10⁹ packed SRBC. Sera were collected after 6 weeks and run on IEF gels. After the run, SRBC were overlaid on the gel. Following the diffusion of the antibodies out of the gel, specific antibodies were detected by the appearance of lysis of the red cells after the addition of guinea pig complement⁷ (Fig. 3). Addition of anti-Ig antiserum

was not necessary. As with antibodies to DNP, the heterogeneity of antibodies to SRBC was also restricted. The four individuals of the clone LG₁₅ had three bands in common. After a long exposure to complement, two more shared bands appeared (not shown). The IEF patterns of this clone differed from that of the three individuals of clone LG₁₁. Within clone 14, animals 1 and 2 shared two bands, as did animals 2 and 3.

In view of the antigenic heterogeneity of SRBC as compared to DNP, it was surprising that *Xenopus* responded in such a restricted way. Thus the existence of an extra clonotype (individual no. 3 of clone LG₁₅, Fig. 3) was by no means surprising.

The high coincidence of isoelectric focusing suggests germ line genes coding for the primary anti-sheep red blood cell (SRBC) specificities (which are responsible for the complement dependent lysis).

When the animals had been reinjected, the pattern for anti-SRBC antibodies were more heterogeneous. Even though differences between different individuals of the same clone became more pronounced than in the primary response, many bands were still shared by the different individuals.

Although the primary and secondary response of outbred individuals to DNP and SRBC was heterogeneous (though not as heterogeneous as in mouse) and differed much more from one individual to the other than in the case of isogenetic animals, shared bands were detected (Fig. 4).

Inbred mouse strains show great heterogeneity in their IEF patterns to cross-reactive antibodies to DNP and TNP⁸ to NIP (4-hydroxy-5-iodo-3-nitro-phenacetyl)⁹, SRBC⁷, and of antibodies which reconstitute mutants of the enzyme β-galactosidase to wild-type activity¹⁰. Isogenetic *Xenopus*, however, show a high incidence of coincidence in IEF bands of antibodies in their primary anti-DNP and anti-SRBC responses. Although the occurrence of some somatic changes of germ line genes is not excluded, the concept of somatic generation of antibody diversity in *Xenopus* is not necessary in order to interpret these data. While somatic mutation may be the driving force for antibody diversity in mammals, *Xenopus*, a primitive anuran amphibian, might lack an appropriate mutator mechanism.

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hCG-induced decrease in availability of rat testis receptors

EVIDENCE is slowly accumulating to suggest that hormones may be involved in the regulation of their own receptors in the target tissue¹. The present paper reports findings which suggest that treatment with human chorionic gonadotropin (hCG) may influence the availability of LH/hCG receptors in the immature rat testis. These receptors show a similar high affinity and specificity for LH/hCG to those of the adult rat testis^{2–4}.

Human chorionic gonadotropin was used as in other studies^{3–6} because of its ready availability in purified form with high biological and receptor-binding activity. Its effects on testis LH/hCG receptors was studied in three ways. First, the duration of effect of gonadotropin on testis receptors was determined by killing rats at intervals ranging from 6 to 90 h after hCG injection. Second, the dose-response relationship between hCG and receptor availability was examined. Third, the effect of short term exposure to hCG was studied by removing free hCG from circulation, with an excess of antiserum, at 4 and 8 h after injection of hCG, and then killing animals at 20 and 30 h after the hCG injection. To give a measure of the availability of vacant receptors for LH/hCG, the *in vitro* binding of ¹²⁵I-hCG to homogenates of testes from saline-treated controls and hCG-treated animals was compared. The degree of receptor site occupancy was assessed by injecting 0.5 μ Ci ¹²⁵I-hCG into rats with or without 10 IU hCG, and then measuring the concentration of radioactivity in the blood and testes at intervals between 4 and 40 h after injection.

After injection of 10 IU hCG, serum concentrations, measured by radioimmunoassay⁷, declined from 8 to 40 h and were undetectable (<3 mU ml⁻¹) at 70–90 h (Fig. 1). In the saline-treated controls, serum levels of testosterone, measured by radioimmunoassay⁸ after column chromatography⁹, were undetectable (<54 ng per 100 ml) in all but one animal, whereas hCG-treated rats showed elevated testosterone levels for up to 48 h after injection, with highest values at 6–12 h (Fig. 1). The *in vitro* binding of ¹²⁵I-hCG to testis tissue was not significantly reduced (compared with controls) until 8 h after hCG injection (Fig. 1); thereafter, binding declined to 10% of control levels at 30 h after injection, remained at this low level until 48 h, then progressively recovered towards control levels between 70 and 90 h. Varying the dose of hCG injected (0.1–10 IU) had a

dose-related effect on the *in vitro* binding of labelled hCG at 42 h after injection, and as little as 0.01 IU hCG still significantly reduced binding and caused an elevation of testosterone levels (Table 1). Injection of antiserum to hLH only partially prevented the reduction in binding caused by hCG at 20 and 30 h after injection, and did not significantly affect serum testosterone levels (Table 2).

In vivo, the ratio of radioactivity in the testes to that in the blood (T/B) increased significantly ($P < 0.05$) from 4 to

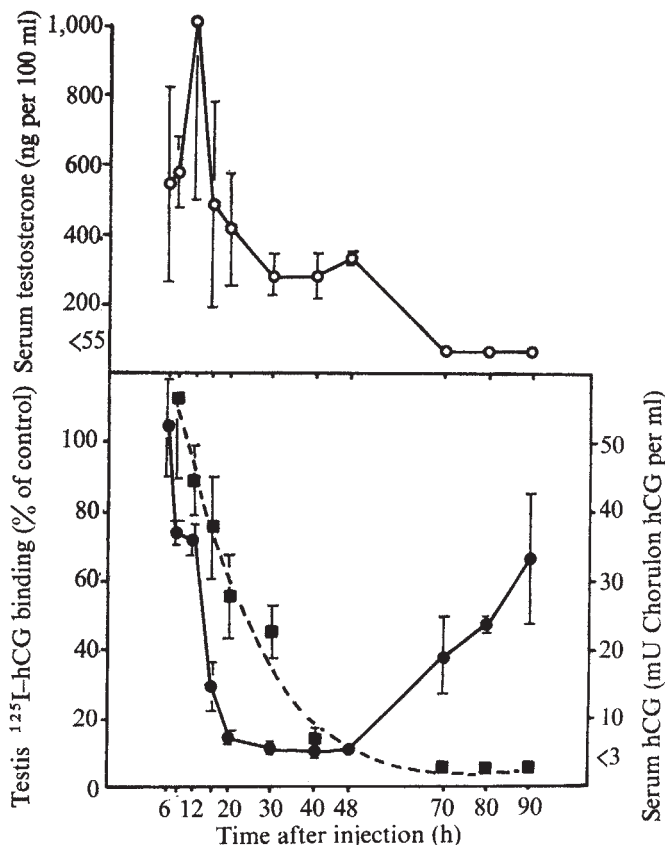


Fig. 1 *In vitro* binding of ¹²⁵I-hCG by testis homogenates in relation to serum levels of hCG and testosterone in hCG-treated immature rats. Twenty-one-day-old L/H rats were injected subcutaneously with either 10 IU hCG (Chorulon) in 0.5 ml 0.9% saline or with the vehicle. Pairs of saline-treated (control) and hCG-treated rats were killed at various intervals after injection. Animals were anaesthetised with ether, blood collected by decapitation and the paired testes removed and decapsulated in Krebs–Ringer bicarbonate solution (KRB). Testes from each individual rat were gently homogenised in 2 ml KRB in a glass homogeniser, filtered through a single thickness of gauze and the filtrate centrifuged at 1,500g for 5 min. The pellet was then weighed and resuspended in KRB at a concentration of 100 mg tissue ml⁻¹ and stored at –20 °C. When all samples had been collected, homogenates were thawed, resuspended and 0.2 ml added to 7 ml polystyrene tubes, which were then incubated in duplicate with ¹²⁵I-hCG (approximately 50,000 c.p.m. in 0.1 ml KRB containing 0.2% bovine serum albumin, fraction V) for 3 h at 37 °C. Non-specific binding of ¹²⁵I-hCG by each homogenate was determined by incubation of tubes containing 100 IU hCG. Incubation was terminated by addition of 5 ml cold 0.9% saline. Tubes were then centrifuged at 1,500g for 20 min, the supernatant decanted and radioactivity in the pellet measured in a gamma spectrometer. The ¹²⁵I-hCG was hCG CR119 (11,600 IU mg⁻¹) iodinated by the Chloramine-T method to a specific activity of approximately 40–50 μ Ci μ g⁻¹; the labelled hCG was purified on a column of cellulose CF11. Nonspecific binding was 0.5–1.6% of the total counts added. Specific binding, computed as the counts bound in the absence of unlabelled hCG minus the nonspecific binding, was 5–6% for control immature rats (20–27% for comparable adult tissue). ●, binding of ¹²⁵I-hCG in hCG-treated rats expressed as a % of the mean binding by tissue from the respective controls. Binding was reduced significantly ($P < 0.05$) at all times other than at 6 h, compared with controls. ○, Serum testosterone; ■, serum hCG. Vertical bars represent ± 1 s.d.

Table 1 Effect of varying doses of injected hCG on the *in vitro* binding of ¹²⁵I-hCG 42 h later

hCG injected (IU)	¹²⁵ I-hCG bound		Serum testosterone (ng per 100 ml)
	c.p.m.	% of control	
0	1,610 ± 178	(100)	< 56
10	193 ± 83	12.0†	166 ± 9
4	468 ± 88	29.1‡	129 ± 83
1	883 ± 249	54.8*	124 ± 38
0.1	1,044 ± 144	64.8†	67 ± 49
0.01	1,000 ± 68	62.1†	95 ± 24

There were three animals per treatment group. Experimental procedures were as described in Fig. 1, except that only 10 mg fresh homogenate per tube was used for incubations.

Values are mean ± 1 s.d. * $P < 0.02$; † $P < 0.01$; ‡ $P < 0.001$, compared with control.

8 h after injection of ^{125}I -hCG, and then remained fairly constant until 40 h (Fig. 2), suggesting that an equilibrium had been established. Injection of 10 IU hCG apparently disrupted this equilibrium, initially stimulating the uptake of ^{125}I -hCG and then lowering it (Fig. 2). Receptor occupancy, as judged by the actual level of radioactivity in the testes, increased between 4 and 8 h after injection but then fell by over 90% between 8 and 40 h in both treatment groups (data not shown). Labelled hCG in the blood showed a similar disappearance curve to that shown for hCG in Fig. 1.

Reduced binding of ^{125}I -hCG to testis tissue from hCG-treated rats could be due simply to saturation of the receptors by injected hCG; in this case one would expect an inverse relationship between binding of ^{125}I -hCG *in vitro* and the degree of receptor occupancy. This expectation does not match the findings, as receptor occupancy and the binding of ^{125}I -hCG *in vitro* decreased together between 8 and 40 h after injection of hCG. This suggests that hCG has caused a reduction in the actual number of hCG-receptors in the testis. Again, findings *in vivo* show a distinct difference in the ability of the testis to bind ^{125}I -hCG before and after exposure to hCG. In the first 8 h after injection, hCG stimulated the *in vivo* uptake of ^{125}I -hCG, probably by increasing testicular blood flow¹⁰; in contrast, at 40 h, when uptake had fallen well below the maximum, further injection of hCG/ ^{125}I -hCG failed to increase uptake of the labelled hormone 8 h later ($n=3$; saline-treated at 40 h, $T/B=0.81\pm0.21$; hCG/ ^{125}I -hCG-treated, $T/B=0.60\pm0.06$). This finding would seem to confirm that exposure to hCG results in impairment of testicular hCG binding by reducing the availability of receptors. Furthermore, as this effect is less pronounced following short term treatment with hCG, and is dose dependent, it seems likely that the extent of receptor loss is determined by the degree of exposure to hCG.

The observation that both short and long term exposure to hCG stimulated testosterone release to a similar extent may mean that the steroidogenic response of the testis is restricted to the first 12 h or so after injection; the gradual decline in testosterone levels between 12 and 70 h after injection of hCG might then reflect clearance of this steroid. Alternatively, the ability of the testis to secrete testosterone may have fallen as a result of the decrease in receptor availability, as animals given two injections of hCG 42 h apart show no significant elevation of testosterone 6 h after the second injection (unpublished data).

Similar results have been reported in the hypophysectomised female rat¹¹, where treatment with hCG or LH caused a reduction in hCG-binding by the ovary *in vitro* and *in vivo* up to 90 h later. Again, ovarian LH/hCG receptors in cultured follicles have been shown to become refractory to further ovine LH stimulation following an

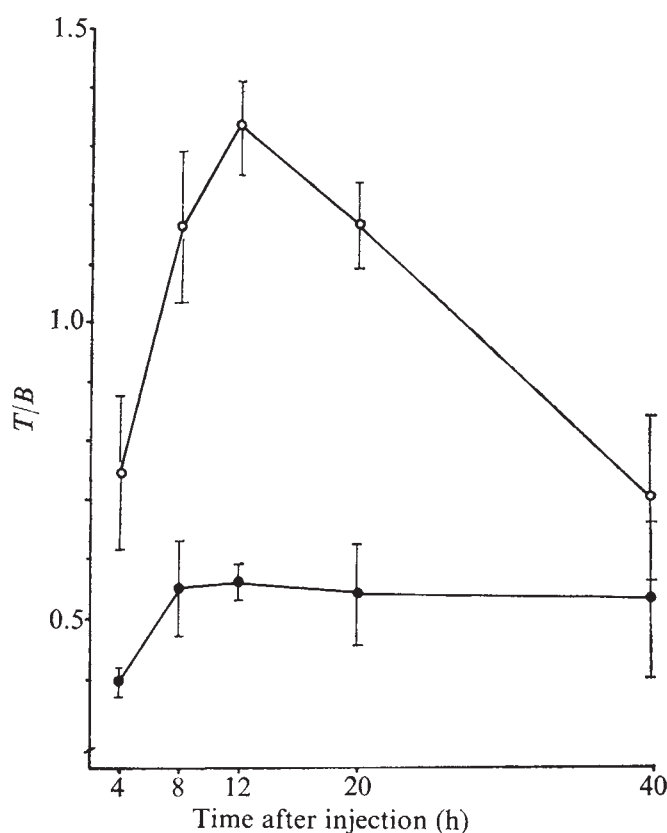


Fig. 2 Effect of injected hCG on the *in vivo* binding of ^{125}I -hCG by immature rat testes. Injections were given subcutaneously in 0.5 ml 0.9% saline. Animals were killed with ether at intervals after injection, 0.2 ml blood obtained by cardiac puncture and the paired testes removed and cleaned of extraneous fat. Blood and testes were placed in tared tubes, weighed, and radioactivity measured in a gamma spectrometer. The ^{125}I -hCG injected was prepared as described in Fig. 1, and retained full biological activity, as judged by the ability of equal amounts of labelled and unlabelled hCG to stimulate similar release of testosterone *in vitro* by decapsulated rat testes⁶. Preliminary experiments showed that, after injection of 0.5 μCi ^{125}I -hCG, the concentration of radioactivity in the testes was between 3.3 and 4.5 times higher than that in muscle at 2–40 h. Results are plotted as the ratio of radioactivity per mg testis over the radioactivity per mg blood. ●, Injected with ^{125}I -hCG only (three to four animals per point); ○, injected with ^{125}I -hCG together with 10 IU hCG (two to three animals per point). Vertical bars represent ± 1 s.d.

initial exposure to ovine LH¹², while LH has also been implicated in the loss of LH-receptors during luteinisation¹³.

These results suggest that binding of hCG to specific gonadotropin receptors in the rat testis results in changes in the subsequent availability of these receptors which, in

Table 2 Effect of injected antiserum to hLH on *in vitro* testis binding of ^{125}I -hCG in hCG-treated rats

Experiment	No. of animals	Treatments		Time when killed (h)	^{125}I -hCG bound		Serum testosterone (ng per 100 ml)
		1	2		c.p.m.	% of respective control	
a	2	Saline	—	20	3,249 $\pm$ 115	—	< 35
	2	Saline	anti-hLH		3,104 $\pm$ 65	—	< 50
	2	hCG	—		462 $\pm$ 2	14.2 $\ddagger$	414 $\pm$ 156
	4	hCG	anti-hLH		1,905 $\pm$ 136	61.4 $\ddagger$	405 $\pm$ 158
b	2	Saline	anti-hLH	30	2,932 $\pm$ 42	—	< 43
	2	hCG	—		342 $\pm$ 28	11.7 $\ddagger$	277 $\pm$ 57
	2	hCG	anti-hLH		905 $\pm$ 117	30.9 $\ddagger$	193 $\pm$ 55

After the initial injection of saline or 10 IU hCG, second injections were given either 4 h (Exp. a) or 8 h (Exp. b) later. All injections were given subcutaneously in 0.5 ml 0.9% saline. Animals were killed at 20 h (Exp. a) or 30 h (Exp. b) after the first injection. Other details are as shown for Fig. 1 except that testis homogenates were used fresh for uptake studies. A 1 : 10 dilution of serum from saline/antiserum-treated rats bound 66.3 $\pm$ 4.9% (20 h) and 56.9 $\pm$ 0.4% (30 h) of added ^{125}I -hLH under radioimmunoassay conditions; serum (1 : 10) from hCG/antiserum-treated rats bound 56.1 $\pm$ 1.0% (20 h) and 51.8 $\pm$ 1.0% (30 h) of added ^{125}I -hLH. Figures are the mean $\pm$ 1 s.d.

$\ddagger P < 0.01$, $\ddagger\ddagger P < 0.001$ compared with respective control.

turn, may limit the steroidogenic potential of the testis, and may represent a way in which a target tissue can regulate its sensitivity to hormonal stimulation¹.

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Interactions of cholesterol with the Na pump in red blood cells

It is well established that cholesterol, by interacting with phospholipids, decreases the permeability and increases the physical resistance of natural and artificial membranes¹⁻³. In contrast, studies of the interactions of cholesterol with membrane proteins have yielded variable results, depending on the experimental conditions and/or the enzymatic activity tested. For example, in cholesterol-enriched mitochondria, an enhancement of activity is seen only in the case of succinate-cytochrome *c* reductase⁴. The activity of certain isolated membrane-bound enzymes involved in ionic transport is affected by the presence of cholesterol. Between 50 and 90% inhibition of the phospholipid-induced (Na⁺ + K⁺) - Mg²⁺ - ATPase activation^{5,6} and complete inhibition of the (Ca²⁺) - Mg²⁺ - ATPase activity⁷ have been reported when cholesterol is added to the phospholipid environment of isolated enzymes. This observation suggested that cholesterol is excluded from the membrane areas containing the transport enzymes. Data concerning the Na pump are also conflicting. In cholesterol-depleted red blood cells, some workers found that the pump-mediated K influx is decreased⁸ whereas, according to others, the pump-mediated Na efflux is increased⁹. Because in physiological conditions these fluxes should be stoichiometrically coupled^{10,11}, the observations are contradictory. In addition, it has been reported that the pump-mediated Na efflux in cholesterol-enriched red cells is reduced for guinea-pig cells¹² but unchanged for human cells⁹. We have investigated the effects of cholesterol depletion on the kinetic properties of the well characterised Na pump of human

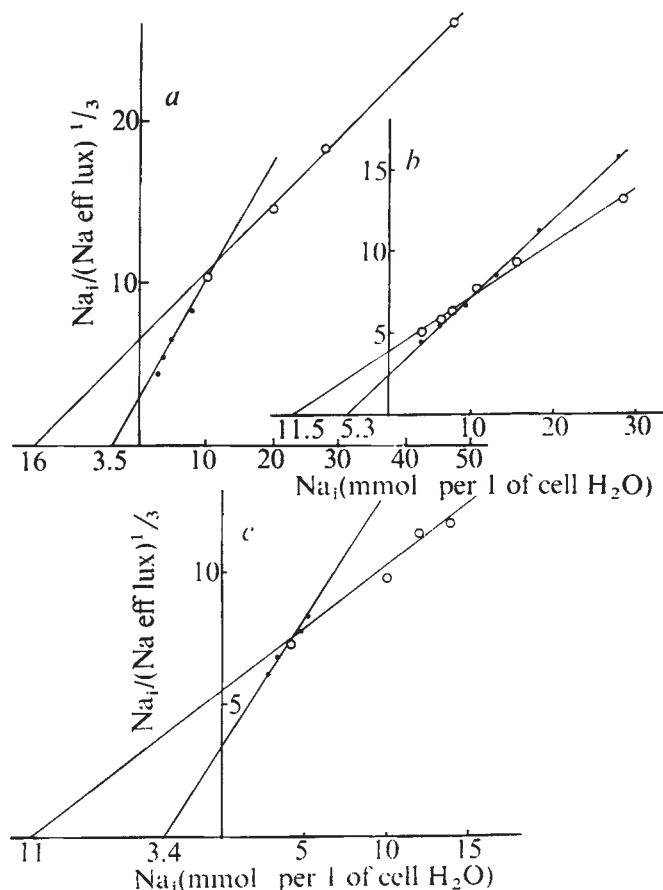


Fig. 1 Effect of internal Na concentration on the ouabain-sensitive Na efflux from control RBC (●) and from cholesterol-depleted RBC (○) plotted according to equation (2). *a* and *b*, Cells were suspended in 10 mM K media (exchange Na-K); *c*, cells were suspended in K-free media (exchange Na-Na). Changes in Na_i were achieved either by incubation at 37 °C in media of different Na concentration (25, 80, 100 and 140 mM) (*a* and *c*), or by treatment with PCMBS and media of various Na concentrations (0, 5, 10, 20, 30 and 50 mM). One typical experiment is shown in each case.

red blood cells (RBC). The turnover of the Na pump and the apparent affinities for internal and external cations were calculated according to a kinetic model described before¹³. Our results show that, depending on the experimental conditions, cholesterol may behave either as an activator or as an inhibitor, thus explaining previous contradictory observations. Moreover, our findings suggest that, in intact membranes, cholesterol is not excluded from the microenvironment of the Na pump.

RBC recovered from freshly drawn human blood were depleted of cholesterol by 15 h of incubation (37 °C, haematocrit 10%) in lecithin vesicle suspensions (L vesicles, 20 mg of lecithin per ml of cells) according to the technique of Bruckdorfer *et al.*¹⁴. Controls were incubated in the same conditions either in mixed cholesterol-lecithin vesicle suspensions (CL vesicles, molar ratio

Table 1 Changes of the RBC membrane lipid content during incubation with lipid vesicle suspensions

	Cholesterol (mg per g of haemoglobin)	Phospholipids (μmol/mol)	Cholesterol Phospholipids (mol/mol)	Cholesterol depletion (%)
Nonincubated cells	3.62 ± 0.20	9.20 ± 0.02	0.77	
Incubated cells				
Controls (CL vesicles)	3.70 ± 0.40	9.16 ± 0.08	0.79	0
Cholesterol-depleted (L vesicles)	2.34 ± 0.28	9.07 ± 0.10	0.51	36 ± 9

Table 2 Effects of cholesterol depletion on the maximal rate of Na efflux ($M_{\max}$) and on the apparent dissociation constant for internal Na (K_{Na}')

	PCMBS	$M_{\max}$ (mmol l ⁻¹ h ⁻¹)	10 mM external K K_{Na}' (mM)	Cholesterol depletion (%)	$M_{\max}$ (mmol l ⁻¹ h ⁻¹)	K-free medium K_{Na}' (mM)	Cholesterol depletion (%)
Controls	—	2.1	3.5	0	1.2	3.4	0
	+	9.7	5.3	10			
Cholesterol depleted	—	14.2	16.0	41	7.1	11.0	28
	+	26.8	11.5	—*			

$M_{\max}$ and K_{Na}' were calculated according to equation (2) from the intercept and slope of the lines of Fig. 1.

* Not measured.

0.85, 4 mg lipids per ml of cells), or in vesicle-free media. Vesicle suspensions were prepared by sonication of either egg lecithin (L vesicles) or cholesterol plus egg lecithin (CL vesicles) for 1 h at 4 °C under nitrogen, and centrifuged for 1 h at 100,000g. Adenine (2 mM) and inosine (10 mM) were added during the last hour of incubation to replenish the energy stores of the cells. The composition of the medium was (mM): (NaCl + KCl), 150; MgCl₂, 1; Na₂HPO₄–NaH₂PO₄, 2.5 (pH 7.4); glucose, 10; penicillin, 5,000 U l⁻¹. Modifications of the internal Na (Na_i) were induced by using media with different Na concentrations during the 15 h of incubation. Alternatively, after the 15 h of treatment at constant external Na, another 12-h incubation was carried out in media with 0.1 mM *para*-chloromercuribenzenesulphonate (PCMBS) and variable Na concentrations¹⁵. Na and K intracellular concentrations and fluxes were measured by a procedure similar to that described by Garrahan and Glynn^{10,11}. Ouabain-sensitive Na efflux was measured as a function of Na_i in control and cholesterol-depleted RBC, either in the presence of 10 mM external K (where the Na efflux is coupled to a K influx: Na–K exchange) or in K-free media (where Na efflux is coupled to a Na influx: Na–Na exchange). As previously reported¹³, the activation curve of Na efflux from red cells can be described adequately by a simple kinetic model. This model assumes that Na translocation will take place only in pump units which have three equivalent and independent sites occupied by Na_i. Therefore the Na efflux (M) obeys the following equation

$$M = \frac{M_{\max}}{(1 + K_{Na}'/Na_i)^3} \quad (1)$$

where $M_{\max}$ is the Na efflux at saturating Na_i and K_{Na}' the apparent dissociation constant of the Na_i-site complex. Equation (1) can be transformed into

$$\frac{Na_i}{M^{1/3}} = \frac{K_{Na}'}{M_{\max}^{1/3}} + \frac{Na_i}{M_{\max}^{1/3}} \quad (2)$$

RBC lipids were extracted in chloroform–isopropanol¹⁶. Cholesterol was measured after Liebermann–Burchard coloured reactions and total lipid phosphorus was determined after removal of water-soluble phosphorus¹⁷.

No significant change was observed in the RBC cholesterol content after incubation in CL vesicle suspensions (Table 1) or in vesicle-free media (results not shown). In contrast, when incubated in L-vesicle suspensions, the cells lost about 36% of their cholesterol (Table 1). In all conditions, the total phospholipid content remained unchanged, so that the molar cholesterol : phospholipid ratio decreased from 0.8 in control to 0.5 in depleted cells (Table 1).

Plots of the function $Na_i/M^{1/3} = f(Na_i)$ (equation (2)) are shown in Fig. 1a and b (exchange Na–K, 10 mM external K) and in Fig. 1c (exchange Na–Na, K-free medium). In Fig. 1a, the range of Na_i obtained without PCMBS, was considerably larger in depleted RBC than in the controls. This agrees with previous evidence that cholesterol removal induced an increase

in the passive permeability of the membrane¹. In contrast, in Fig. 1b, comparable Na_i values were obtained in both types of cell after treatment with PCMBS. In all cases, straight lines are observed, confirming the three-site kinetic model for internal Na, even in situations far from the physiological state, as in cholesterol-depleted membranes. The maximal rate of Na efflux ($M_{\max}$) and the apparent dissociation constant for internal Na (K_{Na}') were calculated from slopes and intercepts of the lines of Fig. 1a, b and c in each experimental condition. In both cases (Na–K and Na–Na exchanges), cholesterol depletion increases the maximal rate of Na efflux (six to seven times) and the apparent dissociation constant for internal Na (three to five times) (Table 2). The kinetic parameters obtained in control cells treated with PCMBS do not coincide exactly with those of cells not so treated (Table 2). This can probably be explained by an additional effect of PCMBS—still to be evaluated—such as a slight decrease in the cholesterol content, as shown in Table 2. Therefore in our experimental conditions cholesterol behaves as an inhibitor of pump turnover and an activator of the apparent affinity for internal Na. The points where the lines cross each other (6–12 mM) define the internal Na concentrations at which cholesterol depletion has no apparent effect on the ouabain-sensitive Na efflux. Below these Na_i values, cholesterol depletion decreases Na efflux whereas above them, it increases Na efflux. It thus seems that an equivalent cholesterol depletion may induce either a decrease or an increase in pump activity. The opposite effects observed by different experimenters would result from differences in the internal Na content.

Since cholesterol alters the apparent affinity of the pump for internal Na, it was interesting to test whether it also affects that for external cations. Apparent affinities were determined by varying external K or external Na at constant internal Na and K. No change in apparent affinities was detected in cholesterol-depleted cells compared with control cells in conditions of either Na–K exchange or Na–Na exchange. This result indicates that the cholesterol effect is specific for the internal sites of the Na pump.

The enhancement of the maximal rate of the Na pump induced by cholesterol depletion may be due to an overall decrease of the internal viscosity of the lipid core of the membrane, as the following observations suggest: (1) addition of cholesterol either in aqueous dispersions of phospholipids or into RBC membranes increases the internal viscosity of the membrane^{18,19}; (2) isolated purified transport enzymes such as (Ca²⁺ + Mg²⁺)-ATPase or (Na⁺ + K⁺)-Mg²⁺-ATPase seem to require a fluid phospholipid environment for optimal activity^{5–7}. This interpretation does not imply that, in depleted cells, cholesterol is removed from the immediate environment of the pump, for a fluidising effect could occur even at a distance. In contrast, a change in the apparent affinity of the pump, as we have detected for internal Na, and which is linked to a conformational change of the protein or to another physicochemical property responsible for the binding, could suggest a direct interaction of cholesterol with the pump. Taking into account the previous experimental evidence for the simultaneous existence of external and internal cation sites on the same pump unit²⁰ and for the quaternary structure of the (Na⁺ + K⁺)-Mg²⁺-ATPase^{21,23}, the specific effect of cholesterol can be interpreted, assuming that

the external and internal cation sites are in different subunits, and that cholesterol interacts only with the internal subunit.

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Anti-actin stains synapses

SMOOTH muscle antibody (SMA) found in the sera of some patients with active chronic hepatitis (ACH)¹ has been shown to contain specific anti-actin antibody^{2,3}. These SMA sera have been used to demonstrate the presence of the corresponding antigen in normal² and neoplastic⁴⁻⁶ 'non-muscle' tissues. We have demonstrated that SMA serum reacts with normal, neoplastic and foetal astrocytes but not with neurone cell bodies^{5,7}. The present report deals with the reaction of SMA with synaptic endings in the central nervous system (CNS).

The neural tissues examined comprised cerebellum and cerebrum from rat, mouse, rabbit, guinea pig, sheep and

chicken, and spinal cord from rat, mouse and chicken. Synaptosome⁸- and myelin⁹-enriched fractions were also isolated from chicken forebrain; effective enrichment was established by electron microscopy and by enzyme marker assays^{8,9}. The fractions were washed three times in phosphate-buffered saline (0.145 M NaCl, 0.01 M sodium phosphate, pH 7.1), resuspended in a minimum volume of the same medium, smeared on glass slides and air-dried at 4 °C for 2 h. The sections and smears were tested with SMA by standard sandwich immunofluorescence (IFL) tests¹⁰.

The SMA serum⁴ from a patient with ACH was characterised by reactivity with the following tissues: smooth muscle¹, skeletal muscle striations^{2,3}, liver in a 'polygonal' pattern², renal glomeruli in a diffuse pattern², the brush borders and peritubular fibrils of renal tubules^{2,6} and thymus medulla². The conjugate used to trace any bound immunoglobulin was a fluorescein-isothiocyanate (FITC)-labelled goat anti-human gammaglobulin with a fluorescein-to-protein molar ratio of 4.0 and a protein content of 0.8 g per 100 ml. Before use, it was appropriately absorbed⁷ so that by itself it gave no staining reaction on test sections or smears. Specificity tests were performed by reacting sections and smears with SMA serum absorbed¹⁰ with pellets of synaptosomal or myelin fractions or absorbed with skeletal muscle actin, myosin, tropomyosin or troponin¹¹. Purity of the isolated muscle proteins was established by polyacrylamide gel electrophoresis¹¹. The precipitate formed by adding SMA to actin was eluted with 0.1 M glycine-HCl, pH 2.8 and the neutralised eluate used in IFL tests³.

In sections of mammalian and chicken cerebellum, the outer molecular layer showed bright staining of fine particles, about 2 µm in diameter (Fig. 1a and b), and the granular layer showed staining of similar particles clustered around ovoid bodies 10-20 µm in diameter (Fig. 1a and c). The white matter (Fig. 1a), the centres of some ovoid bodies (Fig. 1c), and Purkinje cell bodies (Fig. 1a) were negative. Astrocyte staining⁸ was seen in all three layers of the cerebellum but was best visualised between ovoid bodies in the granular layer (Fig. 1a) and in the white matter where its fluorescence was not masked by staining of the bright fluorescent particles. Cerebellar blood vessels were brightly fluorescent. To locate the site of the ovoid bodies in the granular layer precisely, a double immunofluorescence staining technique with rhodamine and FITC was used¹⁰; nuclei were stained with anti-nuclear factor and contractile protein with SMA. This experiment showed that the ovoid bodies were situated between the cell bodies of granule neurones and that the latter did not stain with SMA.

Cerebrum and spinal cord sections showed staining of fine particles in the grey matter indistinguishable from those seen in the cerebellum; the white matter and neurone cell bodies did not stain. Smears of synaptosomal fractions also

Table 1 Immunofluorescence staining of neural and non-neural tissues by SMA

	Staining titre against unfixed frozen sections and/or smears of chicken neural tissues*:					Staining titre against unfixed frozen sections of non-neural tissues:					
	CNS synaptic endings	Synaptosomes	Neurone cell bodies	Astrocytes	Myelin	Smooth muscle in mouse stomach	Rabbit skeletal muscle	Rat liver	Rat renal glomeruli	Rat renal tubules	Rat thymus
SMA											
Absorptions:											
Nil	64	64	< 8	64	< 8	256	64	128	256	64	64
Synaptosomes	< 8	< 8	< 8	< 8	< 8	16	< 8	< 8	16	< 8	< 8
Actin†	< 8	< 8	< 8	< 8	< 8	< 8	< 8	< 8	< 8	< 8	< 8
Myelin	64	64	< 8	64	< 8	256	64	128	256	64	64
Myosin‡	64	64	< 8	64	< 8	256	64	128	256	64	64

* Results with sections of mammalian neural tissues were indistinguishable from those obtained with the chicken.

† Immunoabsorption was carried out by overnight incubation at 4 °C of 0.1 ml SMA serum with 4.0 mg skeletal muscle protein.

‡ Immunoabsorption of SMA serum with 4 mg tropomyosin or troponin also did not reduce the staining titres for neural or non-neural tissues.

showed staining of small (about $2\ \mu\text{m}$) particles (Fig. 1d) while myelin smears were negative.

Immunoabsorption studies (Table 1) showed that SMA staining of neural and non-neural tissues was inhibited by serum absorption with synaptosomal but not myelin fractions and by skeletal muscle actin but not by myosin, tropomyosin or troponin. The neutralised eluate obtained by acid dissociation of the SMA-actin precipitate gave the same staining pattern in neural and non-neural tissues as the original serum.

We conclude that the discrete particles in neural tissues stained by SMA are synaptic endings, based on the following observations: (1) the particle size (about $2\ \mu\text{m}$) in CNS sections *in vivo* and in smears of synaptosomal fractions *in vitro* corresponds to the average diameter of synaptic endings; (2) they are located in the grey matter where synapses are abundant, and not in white matter and (3) particle staining is inhibited by serum absorption with synaptosomal and not myelin fractions. Further, the size of these particles in the CNS is similar to that revealed by staining with antibodies raised in rabbits against chicken synaptic plasma membrane⁸ or synaptic vesicle¹² fractions. The size (about $10\text{--}20\ \mu\text{m}$) and location of the particle-studded ovoid bodies in the cerebellar granular layer, and the inhibition of their staining by serum absorption with synaptosomal and not myelin fractions suggest that these are 'cerebellar glomeruli'—ovoid-shaped structures of about

$20\ \mu\text{m}$ in their greatest dimension, situated between granule neurones, and made up of synaptic complexes of central mossy fibre axons and the dendrites of granule and Golgi neurones¹³. The absence of SMA reactivity with CNS white matter indicates that antigen is not present in neuronal axons or myelin sheaths; the negative staining seen in the centre of ovoid bodies may correspond to the mossy fibre axon around which synapses are formed. The present results show that, in neurones, contractile protein antigen is restricted to synaptic endings and absent from cell bodies and axons; these observations support the results of one ultrastructural study of actin in the CNS¹⁴ but are at variance with those of another¹⁵.

Immunoabsorption studies of SMA serum with subcellular brain fractions and muscle proteins indicate that an actin-like protein is the antigen demonstrated in synaptic endings; this was confirmed by obtaining the same staining pattern with the eluate obtained by acid dissociation of the SMA-actin precipitate. The present results provide immunohistological confirmation for the presence of actin-like protein in synaptic endings—demonstrated previously by actin isolation from synaptosomal fractions of bovine and rat brains¹⁶. In our experience, SMA provides a more precise means of localising contractile protein in synaptic endings than can be obtained with antisera raised in rabbits against brain actomyosin-like proteins¹⁷.

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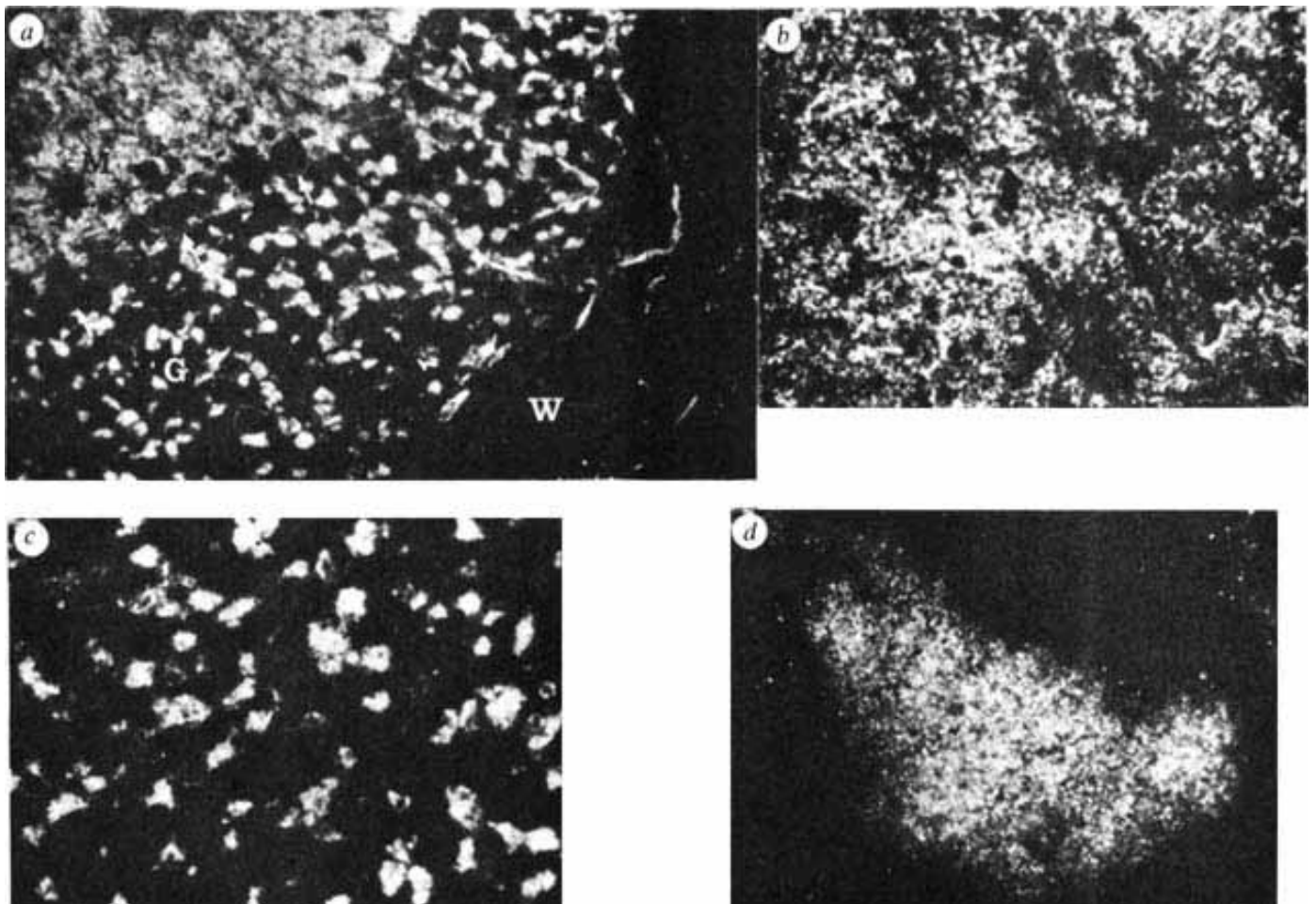


Fig. 1 Indirect immunofluorescence staining of neural tissues by human smooth muscle autoantibody. *a*, Rat cerebellum cryostat section d, showing bright staining of fine particles ($2\ \mu\text{m}$) in the outer molecular layer (M) and of ovoid bodies ($10\text{--}20\ \mu\text{m}$) in the granular layer (G). The white matter (W) and neurone cell bodies are not stained; the negative cell bodies of Purkinje cells are arrowed. The stained cell body and processes of granular layer astrocytes are also seen in this field ($\times 272$). *b*, Chicken cerebellum cryostat section showing fine particulate staining of the outer molecular layer; neurone cell bodies are not stained ($\times 425$). *c*, Chicken cerebellum cryostat section showing fine particles clustered around ovoid bodies of the granular layer; the centres of some ovoid bodies are not stained ($\times 425$). *d*, Chicken synaptosome smear showing fine particulate staining ($\times 425$).

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Hypersensitivity to purified brain proteins in healthy individuals

DURING the course of our research in multiple sclerosis it was observed that apparently healthy laboratory personnel who had been artificially and accidentally exposed to brain tissue showed patterns of hypersensitivity inconsistent with our basic control group. We determined at that time to study them further as a separate group. Twelve people have been identified and studied. We subsequently categorised the types of exposure into three classes as shown in Table 1.

We show here that these individuals develop cellular sensitivity to the brain antigens to which they were exposed, and that the sensitivity is actively blocked in autologous serum by a humoral factor.

The method of lymphoblastic transformation as measured by tritiated thymidine uptake was used throughout. Lymphocytes were isolated from defibrinated peripheral blood by the Ficoll-Hypaque (LSM-Bionetics) method and cultured in RPMI-1640 medium with Hepes buffer, 1% glutamine and penicillin-streptomycin or gentamicin. The antigens used were myelin basic protein (BP) and glial fibrillary acidic (GFA) protein^{1,2} along with control antigens *Candida albicans* (Hollister-Stiers) and purified tetanus antigen. Microtitre plates (Cooke Engineering) were utilised for cultures (400,000 cells in 0.2 ml per well), which were incubated for 6, 7 or 8 d at 37 °C under humid 5% carbon dioxide environment. These cultures were set up in homologous (sex and blood-type matched pools), as well as autologous sera at 10% concentration. The cultures were collected with the Multiple Automated Sample Harvester

(MASH-II, Microbiological Associates) 18-20 h after the addition of 1 μ Ci per well of tritiated thymidine (New England Nuclear). After drying 2 h at 80 °C, the filter paper disks were counted in 5 ml of Liquifluor scintillation cocktail (New England Nuclear) in a Packard liquid scintillation counter. The average counts per minute (c.p.m.) of triplicate determinations were expressed as stimulation index, that is the ratio of average c.p.m. of antigen-exposed cultures to average c.p.m. of antigen-free cultures.

Table 1 Types of exposure

Type	Number	Description
Purification	6	Individuals who were exposed while purifying brain proteins
Autopsy	5	Individuals who were exposed while performing or assisting with autopsies
Injection	1	Individual who accidentally injected self intracutaneously with whole guinea pig myelin homogenised in complete (H37Ra added) Freund's adjuvant

Recognising that individual variations do exist in the temporal response to brain protein (that is, 6, 7, or 8 d of culture), we are presenting only those data obtained on the day of maximum response for each concentration of antigen. The maximum stimulation index for each antigen and concentration was compared with the maximum stimulation index of corresponding antigen and concentration of 13 control individuals who had no determinable history of exogenous exposure to brain or brain components. No significant differences in antigen-free cultures were noted between cells cultured in autologous sera and homologous sera. The data are shown in Table 2.

The most striking observation in the Purification group is the apparent lack of response of these people who have had constant and intense exposure to brains and their

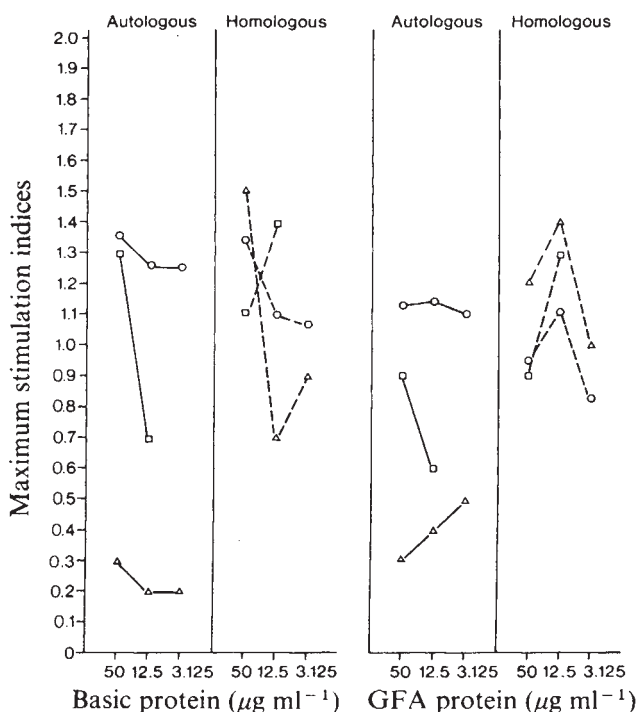


Fig. 1 Stimulation indices of cells from accidentally injected individual at 10 and 38 d post-injection, against Kies myelin basic protein and glial fibrillary acidic protein in autologous and homologous sera. ○, Average stimulation indices of controls; △, average stimulation indices at 10 d post-injection; and □, average stimulation indices at 38 d post-injection. — Autologous sera; --- homologous sera.

isolated component proteins (BP and GFA) when tested in their own sera. The underlying cellular response is unmasked in homologous sera and is significantly increased (dose maxima noted at BP 50 $\mu\text{g ml}^{-1}$ and GFA 12.5 $\mu\text{g ml}^{-1}$). Of great interest is the relative depression in autologous sera (reaching only slight significance, $P < 0.07$), at GFA 50 and titrating back to insignificance at GFA 3.1 ($P < 0.5$). We interpret this to be evidence for active blocking factors in autologous sera. It should be noted that all the subjects in this group are from a single laboratory concerned primarily with the isolation and characterisation of GFA protein.

The Autopsy group, on the other hand, have been exposed primarily to whole brain tissue. Here the basic cellular response (in homologous sera) is clearly significant against BP, and becomes apparent even in autologous sera, suggesting that the blocking factor found in the Purification group may be either decreased or less reactive in the Autopsy group. The response to GFA shows the typical pattern of significantly enhanced response, detectable only in the homologous sera (a slightly enhanced reaction to GFA 12.5 $\mu\text{g ml}^{-1}$ in autologous sera is present, but does not reach significance; $P < 0.09$).

By far, the most intriguing case is the one individual who accidentally injected herself in the finger (6/8/76) with whole guinea pig myelin homogenised in complete (H37Ra added) Freund's adjuvant. At 5 d post-injection she had massive swelling of the finger and hand, but to date has not developed any signs of neurological damage. Injections

Table 3 Average maximum stimulation indices of injection exposure against BP and GFA proteins

Type Injection	Serum	BPC*	Stimulation index† (at 10 and 38 d post-injection)	
			10	38
Auto	Homo	BP 50.0	0.3	1.3
		BP 12.5	0.2	0.7
		BP 3.1	0.2	—
		BP 50.0	1.5	1.1
		BP 12.5	0.7	1.4
		BP 3.1	0.9	—
	Auto	G 50.0	0.3	0.9
		G 12.5	0.4	0.6
		G 3.1	0.5	—
		G 50.0	1.2	0.9
		G 12.5	1.4	1.3
		G 3.1	1.0	—

*Brain protein and concentration; BP is Kies myelin basic protein, G is glial fibrillary acidic protein. Concentration is expressed in $\mu\text{g ml}^{-1}$.

†Maximum stimulation index (see text).

of this sort are known to cause experimental allergic encephalomyelitis (EAE) in monkeys³ and most other mammals⁴ within 2–3 weeks after injection. We first tested her at 10 d (the swelling had subsided almost completely at this time) and subsequently exactly one month later (38 d post-injection) (Table 3 and Fig. 1.) Her initial response to the injected tissue seems to be one of severe depression in autologous serum (note that the cells have normal re-

Table 2 Average maximum stimulation indices of purification and autopsy exposure groups against BP and GFA proteins

Type	Serum	AMRD*	BPC†	AMSI‡	SD	N	Significance§	
Control	Auto	6.8	BP 50.0	1.36	±0.63	10	Not significant	$P < 0.4$
			BP 12.5	1.25	0.28	13		$P < 0.35$
			BP 3.1	1.26	0.51	10		$P > 0.5$
	Homo	7.0	BP 50.0	1.34	0.42	11	Very highly significant	$P < 0.01$
			BP 12.5	1.11	0.30	12		$P < 0.08$
			BP 3.1	1.09	0.45	8		$P < 0.17$
	Auto	6.9	G 50.0	1.13	0.35	10	Slightly significant¶	$P < 0.07$
			G 12.5	1.14	0.46	10		$P < 0.18$
			G 3.1	1.11	0.33	7		$P > 0.5$
	Homo	7.0	G 50.0	0.95	0.33	12	Slightly significant	$P < 0.08$
			G 12.5	1.10	0.37	12		$P < 0.02$
			G 3.1	0.83	0.28	9		$P < 0.16$
Purification	Auto	6.7	BP 50.0	1.73	1.03	6	Significant	$P < 0.02$
			BP 12.5	1.52	0.86	5		$P < 0.06$
			BP 3.1	1.18	0.21	4		$P > 0.5$
	Homo	6.6	BP 50.0	2.92	1.43	6	Slightly significant	$P < 0.06$
			BP 12.5	1.56	0.74	5		$P < 0.04$
			BP 3.1	1.90	1.45	4		$P > 0.5$
	Auto	7.1	G 50.0	0.70¶	0.49	6	Not significant¶	$P < 0.22$
			G 12.5	0.78¶	0.31	4		$P < 0.09$
			G 3.1	1.05¶	0.59	4		$P > 0.5$
	Homo	6.6	G 50.0	1.67	1.23	6	Significant	$P < 0.02$
			G 12.5	1.80	0.61	4		$P < 0.02$
			G 3.1	1.55	1.41	4		$P < 0.16$
Autopsy	Auto	6.5	BP 50.0	2.58	1.06	5	Significant	$P < 0.02$
			BP 12.5	1.80	0.88	5		$P < 0.06$
			BP 3.1	1.60	1.27	2		$P > 0.5$
	Homo	6.6	BP 50.0	4.25	4.85	4	Slightly significant	$P < 0.06$
			BP 12.5	2.15	1.46	4		$P < 0.04$
			BP 3.1	0.80	0.00	2		$P > 0.5$
	Auto	6.8	G 50.0	1.60	1.06	5	Not significant	$P < 0.22$
			G 12.5	1.94	1.24	5		$P < 0.09$
			G 3.1	0.95	0.92	2		$P > 0.5$
	Homo	6.7	G 50.0	1.55	0.41	4	Significant	$P < 0.02$
			G 12.5	1.20	0.35	4		$P < 0.02$
			G 3.1	2.4	—	1		Unknown

*Average maximum response day (all concentrations of protein).

†Brain protein and concentration; BP is Kies myelin basic protein, G is glial fibrillary acidic protein. Concentration is expressed in $\mu\text{g ml}^{-1}$.

‡Average maximum stimulation indices (see text).

§Significance determined by Student's *t* test for two distributions.

¶Note that these are depressions rather than elevations.

activity in homologous serum for both tests) to both BP and GFA proteins. This is followed (at 38 d post-injection) by a tendency toward a "normal" response to BP and a somewhat persistent depression in the presence of GFA protein. Since this individual has worked for 15 yr with myelin basic protein and spinal cord tissue, these reactions probably represent a delayed hypersensitivity reaction in a previously sensitised individual.

We do not know the clinical significance of these results. All of these individuals are healthy and have no apparent symptoms of neurological or other physical problems. We propose that a delicate balance between cellular hypersensitivity and a humoral substance exists in these individuals and that a similar relationship is present in multiple sclerosis patients. The obvious question with respect to multiple sclerosis is: what is the basic difference between these individuals and those who display autoimmune disease? Colby *et al.*⁵ used lymphoblastic transformation against $10 \mu\text{g ml}^{-1}$ of BP at 20% homologous human plasma and reported a temporal relationship of enhanced reactivity to exacerbation. They detected the greatest reactivity within 3 weeks of the onset of exacerbation, while the least was found in patients more than 5 months after an acute episode. Sheremata *et al.*⁶, using macrophage inhibition assay, have evidence that sensitivity can indeed be detected before exacerbation. Our data suggest that an individual without multiple sclerosis will produce blocking factors that suppress the cellular response to exposure (and subsequent damage?) to nervous tissue. It remains to be determined if multiple sclerosis patients are either incapable of producing these blocking factors or produce ineffective ones. It is unclear whether the blocking factors demonstrated in our assay are identical to those observed by Van den Noort and Stjernholm^{7,8}.

At present, we are in the process of increasing our sample size to sort out any possible variables at work in this system: length of time of exposure, severity of exposure (whole brain only as against brain and its proteins), and sustained memory without subsequent exposure.

In conclusion, we would like to emphasise that when control groups are selected in studies involving brain proteins, one must be certain that their backgrounds do not include previous exposure to whole brain or its components, for this would certainly obscure and invalidate the results. Furthermore, when investigating cellular reactivity against different specific antigens, it is highly important to include various concentrations of the specific antigen, to vary the incubation period of the cell cultures and to set up such cultures in both autologous and homologous sera.

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Functional assessment of GABA uptake or exchange by synaptosomal fractions

THE discovery of a transport system in nervous tissue for an amino acid with an apparent K_m equal to or less than 10^{-5} M has been taken as support of a neurotransmitter function for this compound. "High affinity" systems of this sort have been described for γ -amino butyric acid (GABA)¹, glutamate², glycine³, aspartate⁴ and taurine⁵. Initially it was hoped that such transporter systems could be used to map the utilisation of a given transmitter in defined anatomical regions. Unfortunately nonspecific elements, especially glial cells, also have the capacity to transport neurotransmitter candidates using "high affinity" systems⁶. Furthermore, Levi and Raiteri⁷, noting that "high affinity" transport was found where the internal concentration of the amino acid was high, suggested that isotopic measurements of inward transport were misleading and that exchange of labelled amino acid for unlabelled amino acid is likely to be the phenomena that has been measured⁸. This called into question the functional role of "high affinity" transport systems, and has resulted in considerable controversy⁹. In an effort to clarify the conditions of amino acid uptake, we have measured net GABA movement in nerve-terminal-enriched fractions¹⁰.

The conditions were chosen to maximise changes in the extracellular GABA concentrations and mimic as closely as possible the normal *in vivo* ratio of extracellular fluid to cells. Synaptosomes from one rabbit cortex were divided into six equal portions and each was incubated in a minimal volume (200 μl) of Tris-buffered glucose-saline media containing concentrations of GABA in the range 10^{-3} - 10^{-6} M. Experiments were carried out in media containing normal concentrations of sodium and potassium (140 mM Na⁺ and 3 mM K⁺) and in media containing increased K⁺ concentrations (91 mM Na⁺ and 52 mM K⁺), which would decrease both the membrane potential and ionic gradients. The synaptosomes were incubated for 20 min at 37 °C, which was sufficient to achieve equilibria in these conditions. They were sedimented by centrifugation and intra- and extracellular GABA concentrations were measured using GABA transferase and succinic semialdehyde dehydrogenase to generate NADH, which was measured spectrofluorometrically¹¹. The intracellular volume referred to is the portion of the pellet which was not penetrated by ¹⁴C-inulin. The role of GABA metabolism as a driving force was assessed by measuring the

Table 1 Changes in the external [GABA] on incubation of synaptosomes in various concentrations of GABA for 20 min

(GABA) Before Incubation in media (M)	(GABA) After 20 min Incubation	
	in media 10^{-5} M	in synaptosomes 10^{-5} M
10^{-3}	7.0 ± 0.3	111 ± 34
10^{-4}	5.6 ± 0.7	51 ± 17
10^{-5}	2.5 ± 0.4	41 ± 10
10^{-6}	2.1 ± 0.8	19 ± 7

Approximately 100 mg of synaptosomes was incubated in 200 μl of Tris-buffered glucose-saline medium for 20 min. Figures represent initial concentration, the concentration of GABA measured in the media after incubation and the GABA found in the synaptosomes. The synaptosomal content of GABA before incubation was $4.3 \pm 97 \times 10^{-4}$. The total amount of GABA present throughout the experiment was constant. Values are mean $\pm$ s.d. from between three and seven experiments.

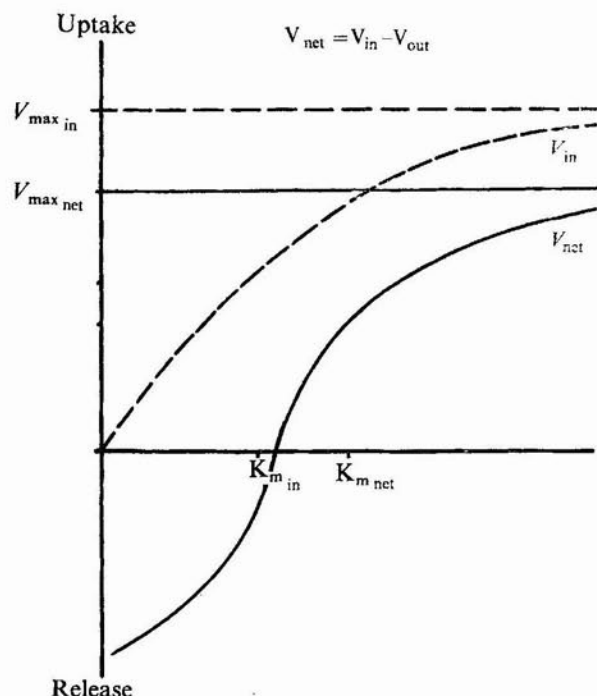


Fig. 1 Uptake or release velocity plotted against substrate concentration for a reversible transport system. This is compared with uptake using isotopic tracers which effectively measure inward flux (v_{in}). This demonstrates that apparent affinity of the substrate and the maximal rate will differ in experiments using isotopic tracer from experiments measuring the actual net rate.

total GABA in the system at the start of incubation and after equilibrium had been reached. The results in Table 1 indicate that GABA degradation is not appreciable and, therefore does not serve as a principal driving force in the system.

These results also indicate that synaptosomes incubated in our experimental conditions release GABA to the media at external GABA concentration of 10^{-6} and 10^{-5} M. At higher external GABA concentrations synaptosomes exhibit concentrative uptake. Thus, in the concentration range in which GABA transport was assessed by following labelled GABA movement there was no net inward transport of GABA. This

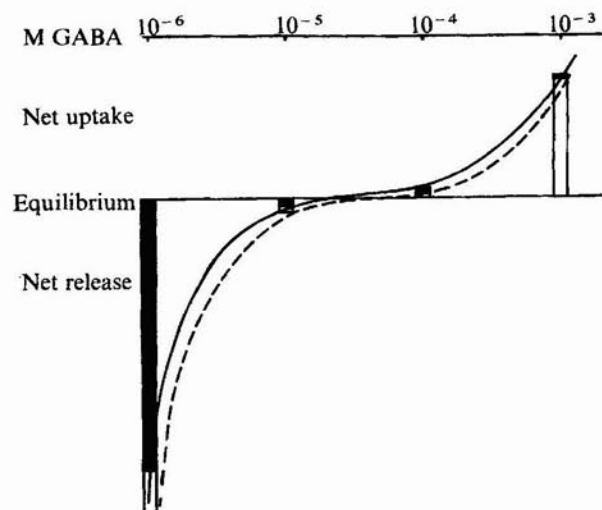


Fig. 2 Equilibrium position of GABA at a variety of initial substrate concentrations. The solid line represents incubation in normal conditions (140 mM Na^+ , 3 mM K^+) while the dashed line represents depolarising conditions (91 mM Na^+ and 52 mM K^+) and illustrates the shift of GABA equilibria effected by reducing the driving force.

situation is illustrated in Fig. 1, which shows the form of plots of velocity against substrate for net GABA movement as opposed to following the inward flux only. Figure 1 also shows that measurements of inward flux made on a reversible transport system result in an apparently increased V_{max} and an apparently decreased K_m , for the inward flux originates at zero substrate concentration while the net flux is zero at the substrate concentration at which the inward and outward fluxes are balanced, thus shifting the curve away from the origin.

Figure 2 is a plot of the equilibrium position for GABA transport in two sets of conditions. The first involved normal uptake media while the second was carried out using a depolarising medium. The alteration in media has the effect of shifting the equilibrium position between net uptake and release. These data suggest that in normal extracellular media no mechanism exists for removing GABA much below 10^{-4} M. This apparent dilemma is resolved by considering quantitatively the factors controlling GABA equilibrium across the synaptosomal membrane. At equilibrium the ratio of internal to external GABA is equal to

$$\frac{[\text{GABA}]_i}{[\text{GABA}]_o} = \left(\frac{[\text{Na}^+]_o}{[\text{Na}^+]_i} \right)^3 \left(\frac{[\text{K}^+]_i}{[\text{K}^+]_o} \right) \exp(-2\Delta\psi F/RT)$$

This assumes that the energy for GABA transport resides entirely in the ionic gradient and that 3 Na^+ and 1 K^+ ion are translocated with each molecule of GABA moved. This stoichiometry fits our unpublished results and agrees with published studies relating GABA transport to ionic fluxes¹³. This equation accounts quantitatively for the shift in GABA equilibrium shown in Fig. 2 when a depolarising medium was used.

Such a correspondence indicates that as a first approximation the forces available in the electrochemical gradient seem to control the distribution of GABA. Because the membrane potential of isolated synaptosomes is considerably less than that of a synaptic ending *in vivo*, we would expect the point at which concentrative uptake begins to shift to lower concentrations considerably. In fact using reasonable estimates for the Na^+ and K^+ gradient and assuming a membrane potential in the range of -50 to -60 mV, it seems that there is sufficient energy in the electrochemical gradient to concentrate GABA more than 100,000-fold in a synaptic ending. From the highest estimates of GABA concentration inside a nerve ending of 100 mM (ref. 14), we can estimate that the balance point at which concentrative uptake begins would be in the range of 10^{-6} M external GABA. This suggests that "high affinity" uptake systems are functionally operative in intact nervous tissue but only detectable kinetically in isolated synaptosomal preparations, which cannot exhibit net uptake in these conditions.

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Strain-dependent variations in number of midbrain dopaminergic neurones

THE activity of tyrosine hydroxylase (TH), the enzyme catalysing the initial and presumed rate-limiting step in the biosynthesis of the catecholamine neurotransmitters dopamine (DA) and noradrenaline (NA), varies over a twofold range in the brains of different strains of inbred mice¹. The molecular mechanisms responsible for these strain-dependent differences in enzyme activity remain to be established^{1,2}.

We have examined the regional differences in TH activity in the brains of BALB/cJ and CBA/J mice. Using immunochemical and immunocytochemical methods, we have investigated: (1) if the difference in TH activity in the whole brains of the two strains is regionally discrete and localised to either the DA or NA neuronal systems; (2) if the difference in enzyme activity can be attributed to variations in either the amount or state of activation of enzyme molecules, and (3) if there are strain-dependent differences in the number of neurones containing the enzyme.

Male BALB/cJ and CBA/J mice (Jackson Laboratories) were killed at 8–10 weeks of age. Brain regions representing sites of high concentration of cell bodies and terminals of DA and NA neurones³ were removed by microdissection. The regions sampled included: (1) the region of the midbrain containing the substantia nigra and adjacent area of the interpeduncular nucleus which includes the cell bodies of the A9 and A10 neurones³ of the nigro-striatal, mesolimbic and mesocortical DA systems⁴; (2) the olfactory tubercle and caudate nucleus, in which TH is localised within DA terminals of the mesolimbic and nigro-striatal systems, respectively^{3,4}; (3) the nucleus locus coeruleus (LC), a region of NA cell bodies; and (4) the hypothalamus, which contains a large innervation of NA fibres and which could be examined by assaying for the activity of dopamine- β -hydroxylase (DBH), a specific biochemical marker of noradrenergic neurones⁵.

Each tissue was homogenised in 10–15 volumes of 5 mM phosphate buffer, pH 6.5, containing 0.2% Triton X-100. After centrifugation, the supernatant was assayed for TH and DBH activity as described before⁶. Immunochemical titration was performed by modification⁷ of the method of Feigelson and Greengard⁸, using specific antibodies for TH prepared from bovine adrenal medulla⁷. For immunocytochemical localisation of TH, four mice from each strain were perfused with 4% paraformaldehyde in phosphate buffer, pH 7.3, postfixed with picric acid-formalin, washed overnight in phosphate buffer and embedded in paraffin. Coronal sections (6 μ m) were taken through the entire brainstem. Sections at 150- μ m intervals through both the substantia nigra-A10 area and the nucleus LC were processed for immunocytochemical localisation of TH by the peroxidase-antiperoxidase method as described elsewhere⁹. The number of TH-containing cells in the midbrain DA neuronal areas (A9 and A10) and in the LC were counted by standard morphometric methods^{6,10}.

We found significant regional differences in the activity of TH in the brains of CBA/J and BALB/cJ mice (Table 1A). The differences were restricted to the DA neuronal systems of both the nigro-striatal and the mesolimbic systems: TH activity was lower by 45% in the substantia nigra-A10 area and by 25–30% in the caudate nucleus and olfactory tubercle in the CBA/J mice compared with the BALB/cJ strain. In contrast, no strain difference in TH and DBH activities were observed in the locus coeruleus or in the DBH activity in the hypothalamus (Table 1B), suggesting that the activity of TH (and DBH) in noradrenergic neurones was similar in the two strains.

We next investigated whether the strain difference in TH activity in midbrain DA neurones was due to a difference in the amount of enzyme protein. Immunochemical titration with a specific antibody to TH demonstrated that the "equivalence point" (the concentration of tissue (enzyme) which just precipitates a fixed amount of antibody and is an indirect measure of the relative amount of enzyme protein in the tissue) of the substantia nigra-A10 area from CBA/J

Table 1 Differences in enzyme activities and cell number in dopaminergic and noradrenergic neurones of two inbred mouse strains

A				
Dopaminergic system: TH activity*				
Strain	Substantia Nigra	Caudate Nucleus	Olfactory Tubercle	
BALB/cJ	2.26±0.07(27)	300.2±5.5(28)	223.4±9.0(8)	
CBA/J	1.25±0.14(14)	226.4±6.6(14)	162.6±7.8(8)	
RATIO†	0.55±0.06‡	0.76±0.02‡	0.73±0.04‡	
B				
Noradrenergic system: TH and DBH activities§				
Strain	Nucleus locus coeruleus		Hypothalamus	
	TH	DBH	DBH	
BALB/cJ	13.5±1.3(13)	0.86±0.11(16)	189.0±7.1(13)	
CBA/J	13.9±1.0(18)	0.97±0.11(10)	197.4±6.4(13)	
Ratio†	1.03±0.09(ns)	0.89±0.11(ns)	0.96±0.04‡	
C				
Cell number and specific activity				
Strain	Substantia nigra		Nucleus locus coeruleus	
	Cell no. (N _t)	Specific activity	Cell no. (N _t)	Specific activity
BALB/cJ	3,384.4±71.3(8)	0.667±0.020	629±41(6)	0.022±0.002
CBA/J	1,664.4±172.5(6)‡	0.751±0.084ns	624±53(4)ns	0.022±0.002ns

Numbers of animals (A and B) and nuclei (C) are given in parentheses. ns, Not significant from BALB/cJ area. Specific activity represents the calculated TH activity per cell.

*Activity expressed as nmol of DOPA formed per region of the substantia nigra-A10 per h and nmol of DOPA formed per g per h for caudate nucleus and olfactory tubercle.

†Ratio = (CBA/J)/(BALB/cJ).

‡ $P < 0.001$.

§TH activity expressed as pmol of DOPA formed per region of locus coeruleus per h. DBH activity expressed as nmol of octopamine formed per region of locus coeruleus per h and nmol of octopamine formed per g per h for hypothalamus.

||Estimated cell number is calculated using the formula $N_t = n_s (S_t/S_s)$, where N_t is estimated total number of cells, n_s is the total number of cells counted in the sample, S_t is the total number of sections through the sample and S_s is the number of sections counted¹⁰.

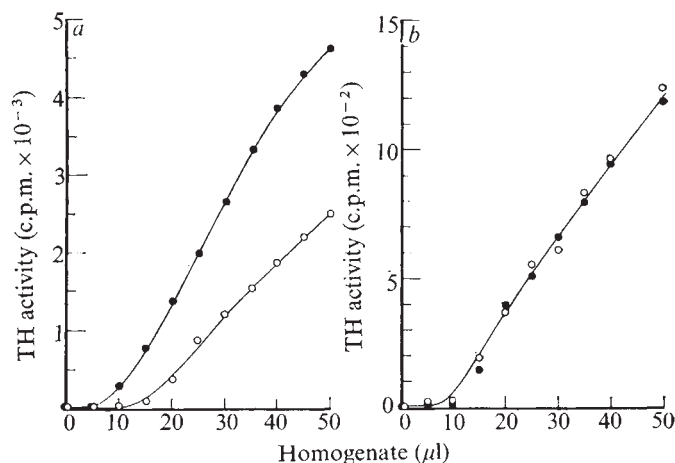


Fig. 1 Immunochemical titration of TH in the (a) substantia nigra-A10 area and (b) locus coeruleus of BALB/cJ (●) and CBA/J (○) mice. Pooled tissues from four mice of each strain were homogenised in 10 volumes (w/v) of 5 mM phosphate buffer pH 7.0, containing 0.2% Triton X-100, and centrifuged. Ten μl of antibody was added to 5–50 μl of homogenate and the final volume was adjusted to 60 μl with buffer. After incubation at room temperature for 1 h, the mixture was centrifuged and TH activity was assayed in the supernatant. In (a) the equivalence point for the substantia nigra-A10 area from the CBA/J mice (13.4 μl) is to the right of that of the BALB/cJ strain (7.0 μl). This demonstrates that the midbrain DA neurones of the CBA/J strain contain 47% less enzyme protein than the identical region of the BALB/cJ strain. The equivalence points for TH in the locus coeruleus (b) from both strains are not statistically different.

mice (13.4 μl) was situated to the right of that of the BALB/cJ strain (7.0 μl) (Fig. 1a). This indicates that the midbrain DA neurones of the CBA/J strain contain 47% less enzyme protein than the identical region of the BALB/cJ strain, which is comparable in magnitude with the difference in enzyme activity. In comparison, the equivalence points for TH of the locus coeruleus from both strains are identical (Fig. 1b), demonstrating that the noradrenergic neurones of both strains contain equivalent amounts of TH enzyme protein.

Catecholaminergic cells within the midbrain and locus coeruleus of BALB/cJ and CBA/J mice were stained immunocytochemically for TH and counted by morphometric methods. TH-containing neurones in the substantia nigra-A10 area of both strains were identified easily. In general they were similar in size, shape and intensity of staining (Fig. 2), although the neuropile of CBA/J appeared to contain more stained DA fibres. There were approximately 50% fewer TH-containing cells within the substantia nigra-A10 area of the CBA/J strain compared with the BALB/cJ mice (1,664 compared with 3,384), a difference comparable with the variation in TH activity (Table 1c). But no strain difference was evident in the calculated specific activity of TH (enzyme activity per neurone) in the midbrain DA neurones of these two strains. Thus, the strain-dependent difference in TH activity in midbrain DA neurones is not the result of a change in the specific activity of the enzyme, but a difference in the total number of DA neurones in this region.

In contrast to the DA system, the number of TH-containing neurones and the specific activity of TH in the nucleus locus coeruleus of these two strains were similar (Table 1c). It is interesting that the TH activity per cell of DA neurones is 30 times greater than that of NA neurones, substantiating the indirect evidence of other workers that the specific activity of TH is greater in DA than in NA neurones¹¹.

Our results show that the twofold difference in the activity of TH in brains of BALB/cJ and CBA/J mice¹ is explained entirely by a difference in the activity and amount

of enzyme within DA neurones. This difference in amount of enzyme protein, in turn, seems to be a consequence of a strain-dependent difference in the number of TH-containing neurones located within the substantia nigra-A10 area of the midbrain comprising the cell bodies of nigro-striatal, mesolimbic and mesocortical DA systems^{3,4}. The fact that the activity of the enzyme per neurone (that is, the specific activity) was the same in DA neurones of both strains, as were the number and specific activity of TH in the noradrenergic neurones in the locus coeruleus, makes it unlikely

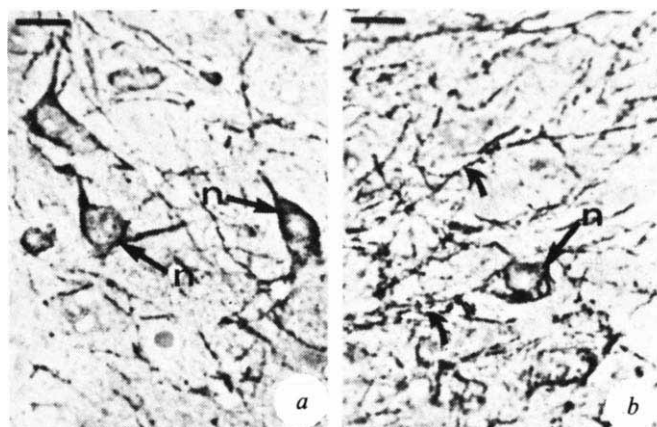


Fig. 2 Immunocytochemical localisation of TH in substantia nigra-A10 area by peroxidase-antiperoxidase method. a, In BALB/cJ strain, the neuronal perikarya (n) show selective cytoplasmic localisation of the enzyme. b In CBA/J strain, the neuronal perikarya (n) are labelled selectively for TH. The neuropile shows an especially dense plexus of labelled fibres (curved arrows) compared with BALB/cJ strain. The bars represent 50 μm.

that variations in staining could account for the observed difference in cell number. But whether the CBA/J mice have fewer DA cells, or whether such cells are present but have too little TH enzyme to be visualised by this method, cannot be answered by the techniques we used.

Although the activity of TH differs by 50% in the substantia nigra-A10 area between these two strains of mice, there is only a 25% difference in terminal fields of the caudate nucleus and olfactory tubercle. This suggests that DA neurones of CBA/J mice have more axonal ramifications per cell, serving to enlarge the terminal fields and compensate for the absolute diminution in cell number. This might also explain the observed increase in the density of TH-staining within the neuropile of the midbrain in this strain.

There is one important clinical implication of this study. It has been postulated that the onset of symptoms in several "degenerative" diseases of the brain, including Parkinson's disease, results from a reduction in the number of specific neurones below a critical number. Thus, individuals with fewer nerve cells may be more susceptible to disease than those endowed with more neurones. This study offers direct evidence that there may be marked, genetically-determined, differences in the number of neurones of a particular chemical class between groups of neurologically normal mice. That DA neuronal systems are affected raises questions about the susceptibility of individuals to Parkinsonism and other proposed diseases of DA neurones, possibly including schizophrenia¹². Thus, genetic variation in the number of neurones within chemically specific cell groups in the brain might be important in influencing the susceptibility to the expression of some diseases of brain.

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Circadian rhythm in cerebrospinal fluid noradrenaline of man and monkey

BRAIN areas with the highest concentration of noradrenergic nerve terminals and noradrenaline (NA) have a circadian rhythm in their content of NA in the rabbit, rat and cat. The anterior and lateral hypothalamus¹ and cervical spinal cord², midbrain³ and caudate nucleus⁴, medial lower brain stem⁵ and frontal cortex⁶ all have significant circadian variations in their NA content. These brain regions have their highest NA level during the night in the rat, a nocturnal animal, and during the day in the rabbit and cat.

NA is present in cerebrospinal fluid (CSF) in measurable quantities⁷⁻¹⁰ and the brain areas having the highest NA concentrations are located in close proximity to the CSF². Levels of NA in CSF are affected by uptake into these brain areas since NA injected into the lateral cerebral ventricle is actively and rapidly taken up into catecholaminergic neurones lying adjacent to the cerebral ventricles^{11,12}. NA levels in CSF should also reflect release from adjacent brain areas as the concentration of NA in brain tissues is typically 1,000 times as high as in CSF⁷. We have measured the NA content of CSF from monkey and man and found a circadian rhythm with NA content highest in the afternoon. This rhythm is persistent even during altered environmental stimulation and lighting.

NA was measured by the radioenzymatic method of Henry *et al.*¹³ as modified for CSF by Ziegler *et al.*⁷. This method enzymatically converts NA to ³H-adrenalin by the phenylethanolamine-N-methyl-transferase catalysed transfer of a ³H-methyl group from ³H-S-adenosylmethionine. The assay could detect 20 pg NA per ml of CSF.

CSF was continuously sampled from four different monkeys on a total of 34 d. The CSF flowed at a rate of 0.8 ml h⁻¹ from a chronically implanted cannula in the lateral cerebral ventricle into a fraction collector kept at 3 °C and set to advance one tube every 3 h. Each collection tube contained 10 mg ascorbic acid to prevent oxidation of NA. NA levels in CSF from the monkeys' lateral ventricle were lowest from 2400 to 0900 and then rose in the morning and plateaued from 1200 until 1500. CSF was collected from the monkeys on weekdays while researchers and technicians were working in close proximity to the animals, on weekends when the monkeys were seen for only a short period by a single person, and while confined

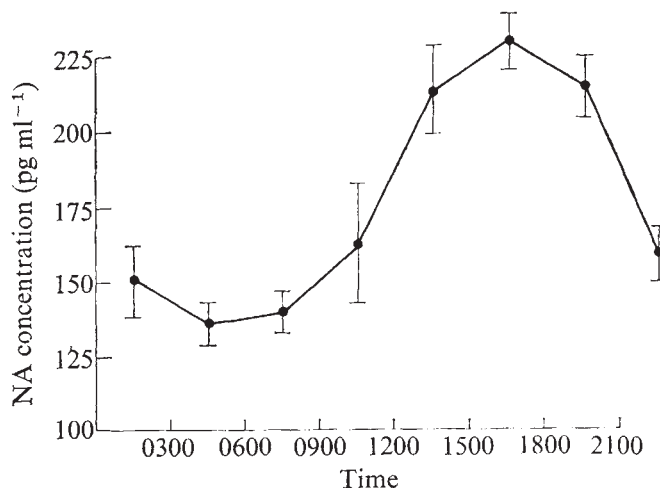
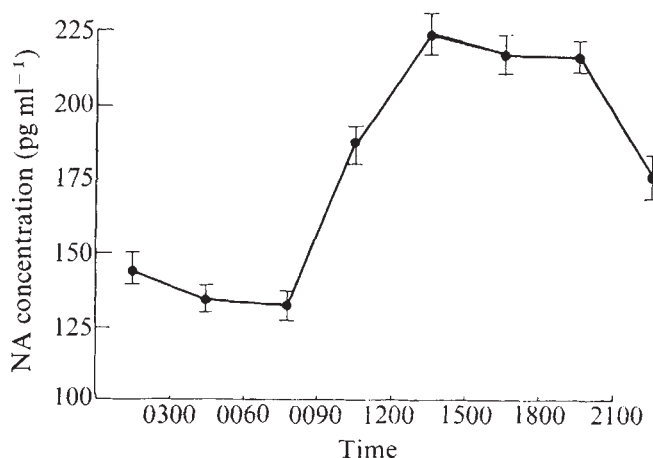


Fig. 1 Concentrations of NA in CSF collected from the lateral ventricle of 4 monkeys on 34 d. Samples were collected over 3 h periods and concentrations are shown as pg ml⁻¹ of NA $\pm$ s.e.m.

to a closed box which was illuminated from 0600 to 1800. Feeding conditions remained constant throughout the entire study with the animals being manually fed only Purina monkey chow no. 5038 twice daily at 0830 and 1330, 7 d a week. Under these three different conditions there was no discernible difference in the circadian rhythm of CSF NA. The mean NA levels in CSF from these monkeys are shown in Fig. 1. One monkey was confined to a box that was dark from 0600 to 1800 and light during the night for 18 d. His CSF NA levels were measured on nine of those days and maintained the same circadian pattern (Fig. 2) and did not detectably vary from the beginning to the end of this reversed light cycle period. The eight means (one from each collection time period) from the regular light cycle (light from 0600 to 1800) were treated as a single vector as were the eight means for the reversed light cycle. The comparison of the regular and reversed mean vectors was made using Hotelling's *t* square test. The *F* value was not significant, *P*=0.18. Even when individual Student *t* tests were performed on each pair of the eight means, no significant differences were encountered.

Twenty-one patients hospitalised at NIH for various neurological disorders had CSF sampled by lumbar puncture for diagnostic purposes. All the patients were confined to strict bed rest for 18 h before lumbar puncture

Fig. 2 Concentrations of NA in the CSF of one monkey kept in an isolation box that was dark from 0600 to 1800 and light during the night. Samples were collected during the first 3 and last 6 d of an 18 d period and values are pg ml⁻¹ of NA $\pm$ s.e.m. of all samples collected during this reversed light cycle.



which was performed at 0300, 0900 or 1500. The patients' physician collected the 16th to 20th ml of CSF removed into a polypropylene tube containing 10 mg ascorbic acid and placed this tube on ice until it was transferred within 30 min to a -70°C freezer for storage. We have previously shown that there is a gradient of increasing NA in the first 12 ml of lumbar CSF removed from adults⁷ but that NA levels do not further increase as more CSF is removed (C.R.L., J.H.W., M.G.Z. *et al.*, unpublished). NA levels in human CSF are significantly lower at 0300 than at 0900 or 1500 ($P < 0.005$, Student *t* test), but the 0900 and 1500 NA levels do not significantly differ (Fig. 3). Several of the patients in this study had seizure disorders and were receiving phenobarbital, phenytoin and ethosuximide; these drugs do not seem to alter levels of NA in CSF. The patients with seizure disorders are randomly distributed throughout the three groups of patients (Table 1). The levels of NA in human CSF seem to parallel monkey NA levels both in concentration of NA and the direction of the circadian rhythm.

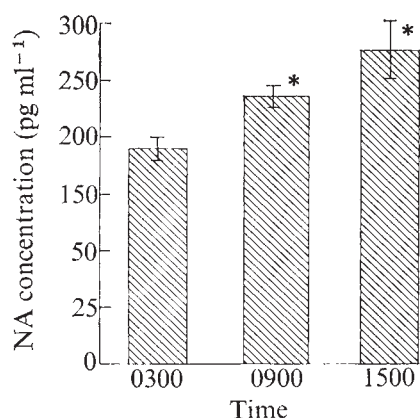


Fig. 3 Levels of NA in the CSF of human patients at 0300, 0900 and 1500. The NA levels of CSF of individual patients are given in Table 1. *Significantly higher than the NA level at 0300 ($P < 0.005$ by Student's *t* test).

NA levels in CSF must represent the amount of NA released into the CSF minus the amount cleared by uptake or chemical conversion. There are several reasons why variations in neuronal release of NA might be the cause of the cyclic variations of NA in the CSF. Levels of NA are highest in CSF during the period when primates are most active, and in depressed patients activity increases the excretion of 3-methoxy-4-hydroxyphenylglycol (MHPG)¹⁴ the major central nervous system metabolite of NA. Noradrenergic nerve terminals in brain areas adjacent to the CSF have their highest NA content in the cat, rat and rabbit when these animals are usually awake¹⁻⁶. Drugs that block NA synthesis or deplete brain NA decrease motor activity¹⁵ and drugs that release NA increase the rate of performance of conditioned behaviour¹⁶. The infusion of NA into the cerebral ventricles increases locomotor activity¹⁷. It seems reasonable that the higher level of NA in CSF of primates during their most active part of the day represents an increased rate of release of NA from nerve terminals. The rhythm of NA in CSF probably does not represent NA synthesis in the entire brain as both the NA content¹⁸ and rate of NA synthesis¹⁹ are highest in rat whole brain during the time of day when rats usually sleep and are least active.

The NA rhythm of the pineal gland is altered by environmental lighting²⁰ but the NA rhythm in the cervical cord and anterior hypothalamus is not changed by an altered lighting schedule³ and is endogenous to the animal.

Table 1 Age, diagnosis, time of lumbar puncture and CSF level of NA in 21 patients

Patient	Age (yr)	Diagnosis	Time	NA in CSF
A	60	Choroid plexus papilloma	0800	210
B	51	Occipital lobe AVM*	0300	182
C	29	Seizures	0300	146
D	18	Seizures	0300	201
E	23	Seizures	0300	203
F	21	Spinal cord AVM*	0300	205
G	67	Cervical spondylosis	0900	234
H	73	Spinal cord AVM*	0900	244
I	57	Temporo-occipital AVM*	0900	212
J	32	Seizures	0900	261
K	51	Extracranial AVM*	0900	274
L	23	Pituitary tumour	0900	225
M	29	Spinal cord AVM*	0900	210
N	63	Dizziness	0900	226
O	16	Seizures	1500	223
P	26	Scalp infection	1500	270
Q	27	Seizures	1500	206
R	36	Seizures	1500	373
S	31	Seizures	1500	325
T	32	Seizures	1500	208
U	48	Headache	1500	326

*AVM = arterio-venous malformation

The circadian rhythm of NA in monkey CSF was not altered by 18 d of a change in light cycle analogous to travel to a point half-way around the world and is apparently endogenous to the animal.

Some drugs that interact with brain NA have variable effects in the rat depending on the time of day they are administered. Reserpine depletes brain NA most effectively in the dark and the mortality rate from reserpine varies with the time of administration in rats²¹. Methamphetamine improves task performance in rats best in the dark¹⁶. These drugs might have their most marked central nervous system effects in man during the day.

Several investigators have measured NA in CSF by fluorometric assays and reported increased levels of NA in patients with stroke²², hypertension²³ and psychiatric disorders⁹. Others have reported abnormal levels of MHPG, the major brain metabolite of NA, in the CSF of patients with depression²⁴, mania²⁵ and schizophrenia²⁶. Further studies of CSF levels of NA and its metabolites should be controlled for time of sampling and activity and in man might best be done in the afternoon when NA levels seem to be relatively constant.

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Motor endplate alterations in schizophrenic patients

NEUROMUSCULAR pathology has been described in a variety of mentally ill patients, primarily those suffering from schizophrenia or the affective psychosis. Some psychotic patients have increased electrical activity of skeletal muscle at rest¹, abnormal electromyograms², perform poorly on various tests of neuromuscular coordination³, have elevated serum creatine phosphokinase activity during an acute psychotic episode⁴ and show an increased incidence of morphological abnormalities of skeletal muscle⁵⁻⁸. Such studies have suggested that there may be an alteration of muscle innervation in such patients. We have previously reported that psychotic patients have an increased incidence of branching and sprouting of intramuscular nerve twigs⁶. We now report significant alterations in the morphology of the nerve terminals at the motor endplate in psychotic patients. This suggests that there may be an abnormality of transmission at neuromuscular synapses in some psychotic patients.

Subjects were 24 in-patients at a psychiatric unit at the Illinois State Psychiatric Institute. Eight were men and 16 were women: their mean age was 28, with a range of 19-42. Each patient was evaluated by two psychiatrists and a psychiatric social worker, and a research diagnosis was established by consensus. All subjects met the criteria for a diagnosis of schizophrenia on the Yale-New Haven Schizophrenia Index. Patient data were compared with results obtained from five normal, healthy volunteers who had no personal or family history of mental illness.

Muscle biopsies of the peroneus brevis muscle were performed on all subjects as described previously⁶. Peroneus brevis was chosen as the biopsy site because the muscle is relatively easily biopsied and there are detailed analyses in the literature of the pattern of methylene blue stained intramuscular nerve endings in this muscle^{7,8}. The subterminal motor neurones were supravitaly stained with methylene blue⁹ and the motor neurone terminal structure analysed according to the methods of Allen et al.¹⁰. Twenty-five to fifty randomly selected nerve terminals from each subject were either photographed or traced with a camera lucida apparatus, enlarged photographically, and measured with a planimeter. Parameters studied were: the

number of terminal bulbs per endplate, N ; the area of the smallest rectangle which could be fitted to the endplate region, A_T ; the total cross-sectional area of the terminal bulbs, A_N ; the mean terminal bulb area, A_N/N ; and the ratio of A_N to A_T or the synaptic index, S.I.

The differences in nerve terminal organisation of patients from those of controls are represented in Fig. 1 and summarised in Table 1. The number of terminal bulbs per endplate, N , was not significantly different between patients and controls. But the size and dispersion of the terminal bulbs was significantly altered in psychotic patients. The mean total area of endplate arborisation, A_T , was both larger and more variable in patients than controls. Similarly, the patient endplate bulb area, A_N , was significantly more variable. The tendency toward a smaller endplate bulb area of greater variability in patients was demonstrated by the significantly smaller average cross-sectional area A_N/N in the patient group. The SI, a measure of the density of neural structures at the endplate is significantly lower in patients than controls, and more variable ($F = 31.48$, $P = 0.008$).

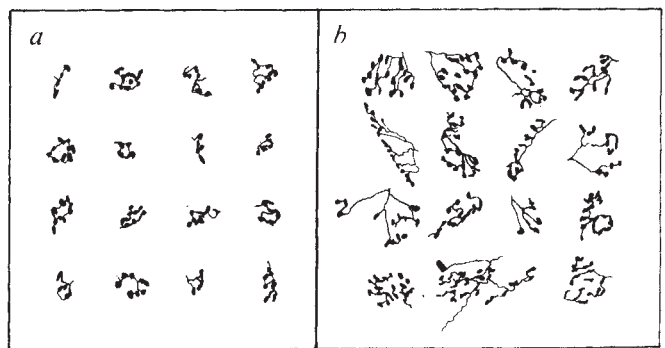


Fig. 1 Comparison of methylene blue-stained motor neurone terminals in muscle biopsy specimens from a normal volunteer control (a) and a schizophrenic patient (b). Biopsies were taken from peroneus brevis muscle.

We also studied the relationship of subterminal nerve branching as assessed by the functional terminal innervation ratio (FTIR) to the terminal arborisation parameters. Figure 2 illustrates a motor neurone from a psychotic patient having extensive branching and increased elaboration of the motor nerve terminals at the endplate. We explored the possibility that only endings on branched neurones would show the endplate alteration, but this did not seem to be the case. In a given patient, if the endplate region was distorted, most endings were affected regardless of whether they belonged to branched or unbranched neurones. The Pearson product-moment correlation coefficient, r , for the relationship between the FTIR and both A_N and A_N/N were significant ($r = 0.3607$, $P = 0.038$; $r = 0.4385$, $P = 0.014$ respectively). Similarly, the FTIR was significantly correlated with A_T ($r = 0.3490$, $P = 0.044$). Surprisingly, the FTIR did not show a significant correlation with the SI. Closer examination of the data revealed that this result was obtained because both A_N and A_T increased as the amount of branching increased; therefore, the ratio A_N/A_T tended to remain constant as FTIR increased. There was, however, the expected trend towards a negative correlation between increased FTIR and reduced SI.

There was no significant difference between diagnostic subtypes such as paranoid and non-paranoid, or acute schizophrenic and chronic schizophrenic, with respect to the parameters studied. In our previous work, we have found muscle pathology in manic-depressive psychotic patients as well as schizophrenic patients. Preliminary observations of three manic-depressive patients suggests that two of three patients have endplate enlargement with a reduced SI. There was no significant correlation between endplate architecture and length of illness, amount or type of psychotropic medication received, or duration of current illness. The endplate abnormalities were

Table 1 Comparisons of control and psychiatric patient endplate histometric parameters

Variable	Control	Patient	P (t test)
N	$6.72 \pm 1.14^*$	7.48 ± 1.31	0.242
$A_T (\mu^2)$	2.53 ± 0.47	3.33 ± 1.70	0.055
$A_N (\mu^2)$	0.96 ± 0.07	0.86 ± 0.38	0.211
$A_N/N (\mu^2)$	0.14 ± 0.01	0.11 ± 0.04	0.005
SI	0.40 ± 0.05	0.28 ± 0.06	0.001
FTIR	1.11 ± 0.05	1.40 ± 0.32	0.001

*Mean $\pm$ s.d.

not age or sex related. The fact that only one of our subjects was over 40 yr of age would tend to rule out the possibility that changes in synaptic indices were due to ageing, since all previously described age changes in neuromuscular structure begin to occur only after the sixth decade of life¹¹.

Nonspecific nerve injury or trauma is a possibility to be considered. There are no previous descriptions of endplate reactions such as those described here to trauma. There are descriptions of smaller terminal arborisations of regenerating neurones in non-psychotic subjects¹². Furthermore, an increased incidence of muscle fibre abnormalities, such as scattered atrophy and fibre type grouping has been seen in biopsies of more proximal muscles in psychotic patients (such as biceps brachii and vastus lateralis), which might be expected to show fewer nonspecific signs of neuronal degeneration in response to age or trauma^{13,14}.

Thus, the endplate region in psychotic patients tends to be larger, and with less nerve terminal area per unit of endplate area. This pattern resembles changes described in myotonic dystrophy¹⁰ and the neuropathy associated in some patients with coeliac disease¹⁵. Since our patients also had increased collateral branching of motor neurones, the possibility exists that the terminal alterations described here are related to the process of regeneration by which motor neurones restore function to previously denervated fibres. This would suggest that psychosis is associated with a neuropathic process with destruction of terminal motor neurones followed by compensatory structural alterations.

It seems likely that the structural alterations noted here are associated with some functional abnormality, although no relevant studies of neuromuscular transmission in psychotic patients have as yet been reported. Kuno *et al.*¹⁶ have shown that spontaneous and evoked acetylcholine (ACh) release at the neuromuscular synapse is proportional to the size of the endplate area. Since endplates in psychotic patients tend to be larger than in normals, ACh release might be expected to be greater than normal. However, the total area of the terminal bulbs, A_N , is smaller in the patients. Since the resultant decrease

The significance of these findings for our understanding of the psychotic process remains to be elucidated. The abnormalities in the subterminal nerve may be a reflection of altered central nervous system physiology such as the putative defect in dopamine metabolism thought to be present in schizophrenic illness. We have recently found (unpublished) that motor neurone excitability as measured by spinal monosynaptic reflex testing is altered in some psychotic patients. The excitability changes are interpretable in terms of altered, descending supraspinal influences. A second possible way to relate these findings to the pathophysiology of psychosis is that the altered motor neurone terminals may reflect a widespread neuronal abnormality that affects neurones in many parts of the nervous system. In either case, the neuromuscular junction seems to be a relatively accessible and potentially rewarding site for studying synaptic neurophysiology in the mentally ill.

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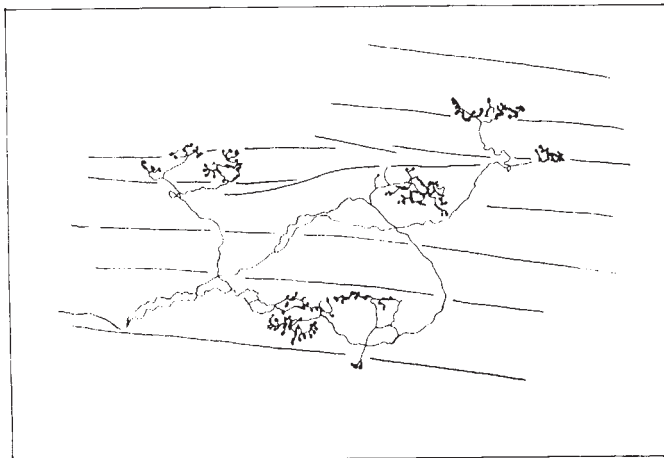


Fig. 2 Extensive terminal branching of a motor neurone in a muscle biopsy specimen from a schizophrenic patient. Normally, the intramuscular nerve ending does not branch, innervating only one muscle fibre. Here eight muscle fibres are innervated by the single neurone. The endings show increased elaboration and enlargement.

in terminal bulb surface area is proportional to the square of the radius of the bulb, there is a proportionately greater reduction in surface area than is represented by the reduction in cross-sectional area of the bulbs. These considerations suggest that spontaneous or evoked ACh release at the neuromuscular synapse in psychotic patients may be abnormal, but the pattern of abnormality would be difficult to predict *a priori*. Conceivably, the abnormal pattern could contribute to the development of pathological morphology in skeletal muscle fibres.

Brain and retinal damage from lathyrus excitotoxin, β -N-oxalyl-L- α , β -diaminopropionic acid

HUMAN consumption of lathyrus peas, especially during periods of famine, has long been associated in certain parts of the world with outbreaks of neurolathyrism, a crippling neurological disorder characterised by spastic paraplegia¹. A neuroexcitatory² amino acid, β -N-oxalyl-L- α , β -diaminopropionic acid (ODAP), isolated from the seeds of *Lathyrus sativus*³ is suspected to be the responsible neurotoxic principle. Although paralysis of the lower extremities has been reported in adult monkeys after lumbar intrathecal administration of ODAP⁴, and similar symptoms have been induced in newborn chicks by intraperitoneal administration⁵, evidence for central nervous tissue damage from systemically administered ODAP has been lacking. We now report that ODAP, administered intraperitoneally to immature mice, induces lesions in the retina, hypothalamus and lower medulla. This pattern of damage is similar to that demonstrated in animals after oral or subcutaneous administration of glutamate (Glu).

ODAP resembles Glu in molecular structure and is a neuroexcitant² and convulsant⁶ similar to but more potent than Glu. When given either subcutaneously⁶ or orally⁷ to infant rodents,

Glu rapidly destroys nerve cells in the retina or select regions of the brain. Several years ago we explored the molecular specificity of this phenomenon^{8,9} and found that structural analogues of Glu known to have neuroexcitatory properties^{10,11}, including aspartic, cysteic, homocysteic, *N*-methyl aspartic and kainic acids, reproduced the Glu type of neuropathological syndrome; analogues known to be more potent than Glu as neuroexcitants were also more potent as neurotoxins. ODAP was not available for inclusion in our earlier studies but was subsequently synthesised by one of us (C.H.M.).

ODAP was synthesised from the copper chelate of L- α , β -diaminopropionic acid and ethyl oxalyl chloride by the method of Rao *et al*³. The product melted at 206 °C (at which it decomposes), and was free from impurities as evidenced by homogeneity in high voltage electrophoresis at pH 3.5. Elemental analysis showed our final product to be the monohydrate of β -*N*-oxalyl-L- α , β -diaminopropionic acid. The analysis calculated for $C_5H_8N_2O_5 \cdot H_2O$ was C, 30.93; H, 5.15; N, 14.43: that found was C, 30.86; H, 5.28; N, 14.34.

Infant Swiss albino mice 7–8 d old were used. Pilot experiments showed that mice in this age range could tolerate intraperitoneal doses of ODAP up to 0.35 mg per g body weight but tended to convulse to death at higher doses. ODAP was administered intraperitoneally at 0.35 mg per g body weight to 56 infants from 8 litters. Controls consisted of two animals from each litter given an equimolar dose of sodium chloride. All animals were killed under anaesthesia by perfusion fixation 3–5 h after treatment and prepared for combined light and electron microscopic examination of the retina and brain as described elsewhere¹².

ODAP caused a lesion in the retina of infant mice (Fig. 1*a*) which was very similar, if not identical, to the lesion seen after treatment with Glu¹³. In the hypothalamus, the arcuate nucleus is affected preferentially by Glu, and the typical effect, illustrated extensively elsewhere^{7,8,12}, consists of acute swelling of dendrites and cell bodies of neurones, with degenerative changes occurring rapidly in both intra-cytoplasmic organelle systems and neuronal nuclei. Axons terminating in or passing

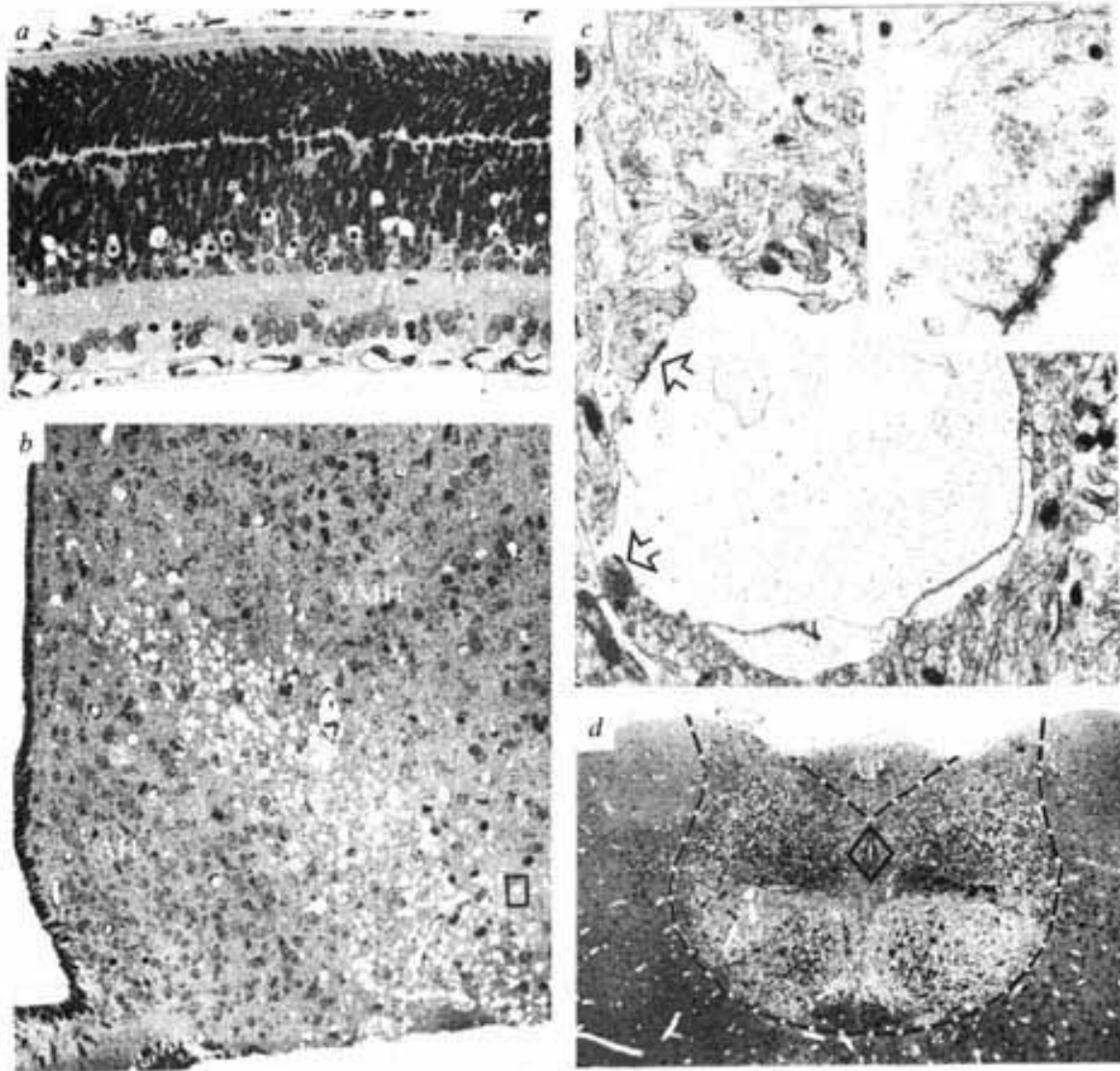


Fig. 1 *a*, A light micrograph of the retina of an 8-d-old mouse 5 h after treatment with ODAP. Acutely necrotic neurones (bull's eye profiles) in bipolar, amacrine and ganglion cell layers are evident, as are dilated dendritic inner plexiform processes ($\times 140$). *b*, A survey electron micrograph of the arcuate (AH)-ventromedial (VMH) hypothalamus of an ODAP-treated neonatal mouse, depicting a band of dilated dendritic processes interposed between arcuate and ventromedial nuclear zones. Scattered ventromedial nucleus neurones appear as dark shrunken profiles. *c*, Swollen, degenerate process from boxed region in (*b*) which is identified as a dendrite by the two synaptic terminals (arrows) impinging on its surface ($\times 8,000$). Inset, magnified view of synapse at upper arrow ($\times 22,000$). *d*, Light micrograph of section at brain stem-spinal cord junction to illustrate the extent and pattern of tissue reaction to ODAP. The area postrema (AP) is relatively unaffected but other regions within a chalice-shaped zone (broken outline) about the central canal (marked by a diamond) are damaged. Numerous oedematous dendritic processes give the lesion a rarefied appearance against which dark, shrunken neuronal cell bodies stand out. The latter are also present in scattered distribution beyond the margins of the oedematous zone ($\times 40$).

through the arcuate nucleus are characteristically spared. Treatment with ODAP resulted in acute histopathological changes in the general vicinity of the arcuate nucleus but the pattern of lesions was different from that associated with Glu treatment. ODAP induced marked swelling of a regionally distinct band of dendritic processes confined to the cell-poor zone between the ventromedial nucleus and arcuate nucleus but had little or no effect on tissue components within the arcuate nucleus proper (Fig. 1b). Processes affected by ODAP were identified as dendritic on the basis of axodendritic synaptic boutons impinging on their surfaces (Fig. 1c). The toxic reaction was restricted to the dendritic (postsynaptic) component of the synaptic complex with sparing of the presynaptic (axonal) component. Dark cell changes, a phenomenon often encountered as a minor feature of Glu neurotoxicity, was relatively more pronounced in the ODAP reaction and the cell bodies affected in the hypothalamus were almost exclusively neurones of the ventromedial nucleus. Although the significance of such changes remains obscure, it is interesting that they were a consistent concomitant of ODAP treatment but were not observed in controls.

Degenerative changes have been observed in the area postrema after oral¹⁴ or subcutaneous¹⁵ administration of Glu to infant mice. The brain stem of mice treated with ODAP in this study was damaged at the level of the area postrema and the extent of damage to the brain stem was relatively greater for any given animal than the damage sustained in the hypothalamus. Typically the lesion induced in the area postrema by Glu is confined to neural elements within the area postrema proper. The pattern of damage induced by ODAP was different; the reaction began at the margins of the area postrema, spared the area postrema proper and tended to spread caudally down the spinal cord in a circular pattern about the central canal (Fig. 1d). A spreading lesion by ODAP at this level in the neuraxis might account for the spastic paraplegic manifestations associated with human ingestion of lathyrus peas containing ODAP. Accordingly, the lesion induced by ODAP at the cervical spinal level warrants more detailed neuropathological investigation.

Here we have shown that systemically administered ODAP has potential for damaging the tissue of the central nervous system. The pattern of distribution of lesions (retina, arcuate nucleus and area postrema) and the dendritic (postsynaptic) locus of the toxic interaction makes the ODAP reaction sufficiently similar to Glu neurotoxicity to consider ODAP an excitotoxic amino acid, that is, one of the structural analogues of Glu which possesses both the neuroexcitatory and neurotoxic properties of Glu and which, therefore, may owe its neurotoxic activity to its neuroexcitatory properties.

ODAP [$\text{CO}_2\text{H}-\text{CO}-\text{NH}-\text{CH}_2-\text{CH}(\text{NH}_2)-\text{CO}_2\text{H}$] is not as closely related in molecular structure to Glu [$\text{CO}_2\text{H}-(\text{CH}_2)_2-\text{CH}(\text{NH}_2)-\text{CO}_2\text{H}$] as are other straight chain excitotoxic amino acids. This may account for certain differences noted between the patterns of lesions caused by ODAP and Glu. Whether such differences reflect dissimilar processing of the two compounds by blood brain barriers or differential binding at receptors within affected brain regions awaits further study. The conformational constraints of the ODAP molecule, however, might be such as to cause it to interact more selectively than Glu at central excitatory receptor sites. It is also possible that other species of receptors, for example high-affinity uptake receptors which may serve a protective function in brain¹⁶, respond differentially to ODAP and Glu. Balcar and Johnston¹⁷ have reported evidence suggesting that ODAP has little or no affinity for receptors of the Glu high-affinity uptake system. All such factors, as well as species, age and nutritional differences between famine-stricken man and the healthy neonatal mouse—plus individual differences among humans, especially in blood brain barrier characteristics—must be taken into consideration in attempting to understand how ODAP causes neurolathyrism. Whether ODAP, like Glu^{7,14}, can damage the brain when taken orally should also be ascertained.

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Persistence of prolonged light-induced conductance change in arthropod photoreceptors on recovery from anoxia

For a wide variety of photoreceptor cells, it has been shown that light alters the conductance of the cell membrane, giving rise to the voltage response of the cell^{1–4}. It is generally accepted that the primary event in the photo-excitation process is a light-induced isomerisation of the photopigment chromophore. The mechanisms linking this isomerisation to the conductance change remain unknown. It has been suggested recently that the transduction process may depend on oxidative metabolism. Intracellular recordings from photoreceptors of the honeybee (*Apis*)⁵, horseshoe crab (*Limulus*)⁶ and barnacle (*Balanus*)—observed recently by one of the authors (A.M.) in collaboration with R. Lantz—have shown that the light-induced conductance increase during illumination can be reversibly blocked by anoxia. Neither the site nor the mechanism of the action of anoxia on the transduction process is known. The light-induced spectral transitions of the photopigments are known, however, to take place under a variety of conditions including anoxia^{6–10}.

In photoreceptors of many arthropods, an intense, coloured stimulus is known to induce a prolonged depolarisation which far outlasts the stimulus^{11–14}. This phenomenon has been termed the prolonged depolarising afterpotential (PDA). In the compound eye of *Drosophila*, an intense blue stimulus ($\lambda=470\text{ nm}$) which shifts a substantial fraction of the photopigment, rhodopsin, ($\lambda_{\text{max}}=480\text{ nm}$)¹⁵, to its thermally stable photoproduct, metarhodopsin, ($\lambda_{\text{max}}=580\text{ nm}$), induces the PDA¹⁶. The PDA so induced decays rapidly if metarhodopsin is photoregenerated to rhodopsin. In *Balanus*, the ionic mechanisms underlying this prolonged afterpotential have been found to be the same as those underlying the normal, light-coincident photoreceptor voltage response¹⁷. Furthermore, from the analysis of the voltage noise superposed on the photoreceptor potential of a *Drosophila* mutant, it has been suggested that the PDA is produced by a summation of a large number of discrete potential fluctuations, just as the light-coincident receptor potential is¹⁸. In this work we studied the effect of oxidative metabolism on the PDA phenomenon in the hope that we might gain some insight into the excitation process in photoreceptors.

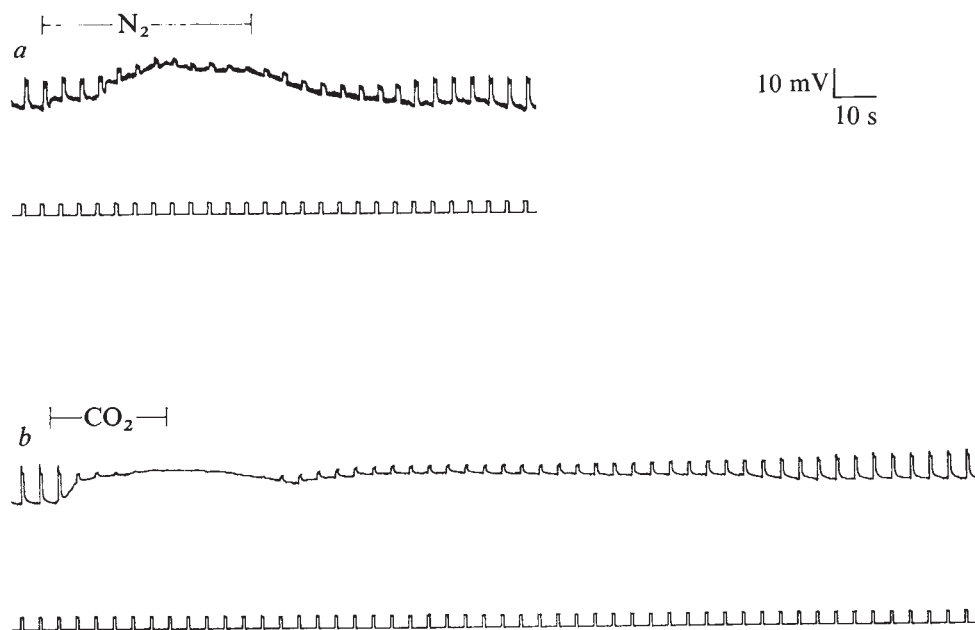


Fig. 1 In these intracellular records, the stimuli were steps of orange light ($\lambda = 600$ nm) at the highest available intensity. For experiments shown in this and the following figures, the light source consisted of a 150-W xenon arc lamp and a Bausch and Lomb high-intensity monochromator. The illuminance at the level of the preparation was about 3×10^{14} photon $\text{cm}^{-2} \text{s}^{-1}$ at both 470 nm and 600 nm. *a*, Effect of N_2 ; *b*, response when CO_2 was used. In both cases, the membrane started to depolarise and the photoresponse diminished soon after the introduction of N_2 or CO_2 . The time course of recovery is different for the two cases. The resting potential of this cell was 27 mV. The horizontal lines indicating the time when N_2 or CO_2 was applied also indicate the zero level for the potential.

Our experiments consisted of intracellular and extracellular recordings from intact preparations of the white-eyed strains of *Drosophila*. Techniques for both intracellular¹⁹ and extracellular²⁰ recordings have been described in detail elsewhere. The animal was placed inside a partially

sealed chamber and either N_2 or CO_2 was allowed to flow into the chamber, lowering the O_2 tension inside the chamber by displacement. The N_2 or CO_2 exerted its effect apparently through the respiration of the animal.

Figure 1 shows the effect of N_2 or CO_2 on the voltage

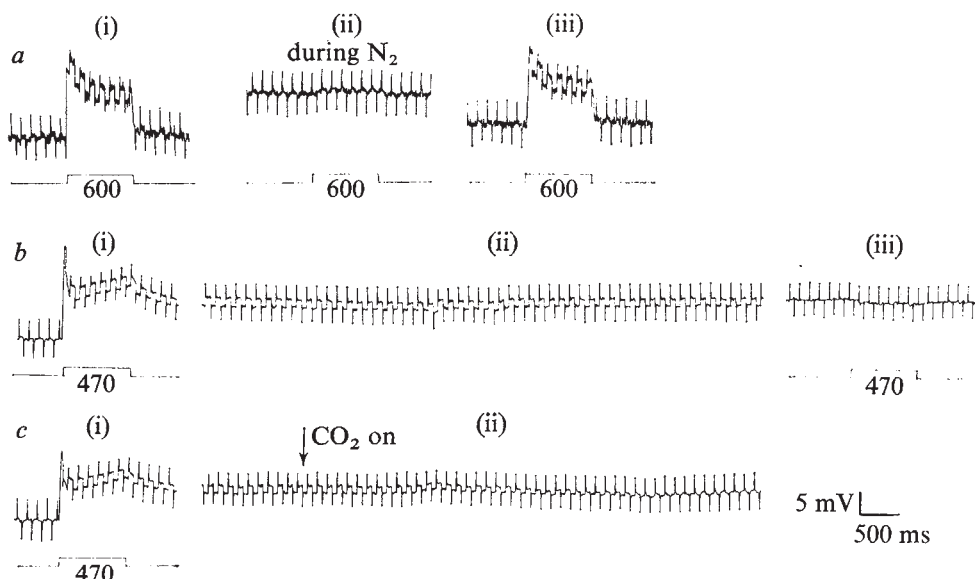


Fig. 2 Intracellular recordings: the conductance was measured by applying current pulses (0.1 nA) to the cell and recording the voltage response of the membrane with a bridge circuit. The bridge was balanced each time before the onset of light. *a*, Trace (i) shows the conductance increase due to a step stimulus of orange light ($\lambda = 600$ nm). The conductance increased only during the stimulus. Trace (ii) shows that during anoxia there was virtually no light-induced conductance change. Trace (iii) shows that the response returned to normal after the animal had recovered from anoxia. *b*, Response to intense blue light. In trace (i), the cell had been previously adapted with orange light. The conductance increased during the stimulus ($\lambda = 470$ nm) but it did not return to the dark value after the stimulus was turned off. This sustained conductance increase is the underlying mechanism for the sustained voltage response (PDA). The beginning of trace (ii) corresponds to 2 s after the PDA was fully induced. Trace (iii) shows the response to a step of blue light during PDA. There was no further increase in conductance change, consistent with the observation that a blue light causes no further depolarising voltage response during PDA. It should be noted that in this trace the bridge was balanced to the conductance during PDA and not to the dark resting value. *c*, We repeated the experiment shown in *b* except that CO_2 was introduced during PDA. The arrow marks the time when CO_2 was introduced. It can be seen that the PDA-associated conductance increase diminished gradually, and after 5 s it returned to the initial resting value (see trace (i)). Comparing trace *c* (ii) with trace *b* (ii), it can be seen that CO_2 abolished the conductance increase. When the cell fully recovered from the effects of CO_2 , no conductance increase could be elicited by a blue stimulus (identical to trace *b* (iii)) showing that the PDA effect had persisted through the period of CO_2 .

response to light. The resting membrane potential started to depolarise soon after N_2 or CO_2 was introduced, and the voltage response to light began to diminish until it was almost completely blocked. The photoresponse returned quickly after N_2 was turned off, and it recovered to its normal value in less than a minute (Fig. 1a). Figure 1b shows the response when CO_2 was used. The general features were the same as those caused by N_2 , but the recovery time of the cell was considerably longer, suggesting that CO_2 might have an effect on the conductance change in addition to that attributable to anoxia. For example, CO_2 might alter the internal pH of the cell which in turn may affect the photoresponse²¹. Once the cell had fully recovered, we could repeat the experiment many times as long as the recording situation was stable. Similar effects of N_2 and CO_2 were also observed in conductance measurements. Figure 2a(i) shows the conductance increase during the stimulus in the absence of N_2 or CO_2 . During anoxia, there was virtually no light-induced conductance change (Fig. 2a(ii)). The response returned to normal after the animal had recovered from anoxia (Fig. 2a(iii)). This suggests that the light-induced conductance change was blocked during anoxia.

Figure 2a(i) also shows that in the cell that had been previously adapted to orange ($\lambda=600$ nm) or white light, an orange stimulus caused the conductance to increase only during the stimulus. If, on the other hand, an orange- or white-adapted cell was exposed to an intense blue stimulus ($\lambda=470$ nm), which caused a substantial net shift of rhodopsin to metarhodopsin, the conductance increased not only during the stimulus but also long after the stimulus was turned off (Fig. 2b(i) and (ii)). This sustained conductance increase is the underlying mechanism for the sustained depolarisation (PDA). The sustained depolarisation lasted more than 30 min after the stimulus was turned off. As seen in Fig. 2b(iii), a blue stimulus caused no further increase in conductance during PDA. It should be noted that in this trace the bridge was balanced to the conductance during PDA and not to the initial dark resting value. The conductance increase during PDA was also sensitive to anoxia and CO_2 . Figure 2c(ii) shows that the conductance increase during PDA, as indicated by the bridge imbalance at the beginning of this trace, diminished gradually after CO_2 was introduced and returned to the initial resting value (see Fig. 2c(i)), 5 s after CO_2 was introduced. Comparing Fig. 2c(ii) with Fig. 2b(ii), it can be seen that CO_2 abolished the conductance increase during PDA. When the cell fully recovered from the effects of CO_2 , it was found that the PDA effect had persisted throughout the period of CO_2 . A blue stimulus was unable to produce a conductance increase just as if CO_2 had not interrupted the conductance increase due to PDA.

In muscoid diptera compound eyes, the maintained component of the electroretinogram (ERG) has been shown to originate primarily from ionic activities of the photoreceptor cells²². Accordingly, most of our experiments requiring stable, long-term recordings were carried out using the ERG. Figure 3a shows the ERG responses to intense, coloured stimuli (470 or 600 nm). If the eye was previously exposed to an intense orange light (Fig. 3a(i)), the response to the first pulse of blue light did not decay (the PDA phenomenon, Fig. 3a(ii)). Subsequent pulses of blue light elicited small responses superposed on the PDA (Fig. 3a(ii)). These superposed responses were previously shown to come from a small population of cells having spectral mechanisms different from the much larger population of cells producing the PDA¹⁹. The first response to an orange stimulus delivered during the PDA is small (Fig. 3a(iii)), but the response recovers in the subsequent pulses of orange light until its recovery is complete in the fourth pulse (Fig. 3a(iii)). The above phenomenon is caused by the reversal of the PDA effect by orange stimuli, which cause

substantial net conversion of metarhodopsin back to rhodopsin. Figure 3b and c show that the above pattern of response to a sequence of orange and blue stimuli is unchanged even if some of the stimuli are applied during the period of anoxia. Figure 3b shows the pattern of ERG responses when the light-induced voltage response was completely blocked by anoxia during the time in which blue pulses were applied. The animal was then allowed to recover from anoxia and was exposed to several pulses of orange light. It may be seen that the responses to orange pulses are small initially but recover in subsequent pulses just as in the case of the control experiment (Fig. 3a(iii)). Intracellular recordings showed that the membrane was in a high conductance (PDA) state when the cell first recovered

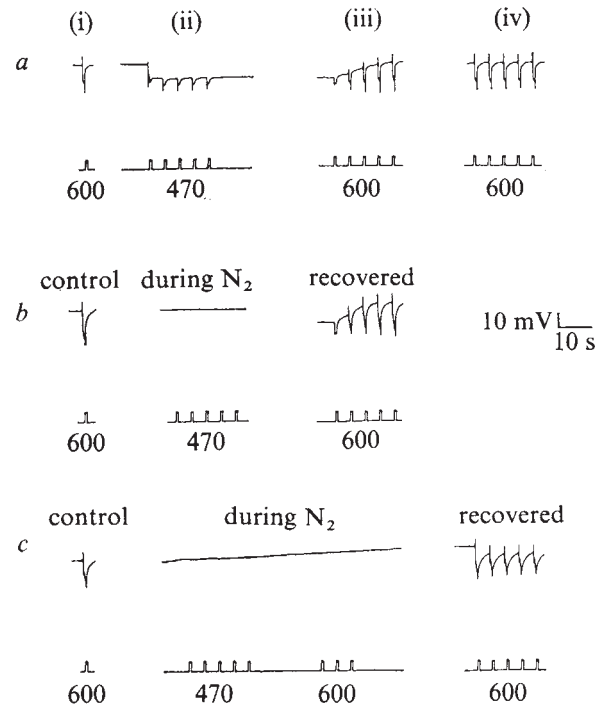


Fig. 3 a, This set of extracellular records shows the ERG responses to intense, blue or orange, stimuli (470 or 600 nm). Trace (i) shows the response to orange light. Trace (ii) shows the response to blue light when the eye was previously adapted with orange light. Note that the first pulse of blue light elicited the PDA. Subsequent pulses of blue light elicited small responses superposed on the PDA. Those responses were previously shown to come from a small population of cells that have a spectral mechanism different from the cells producing the PDA. (See text.) Trace (iii) shows the response to orange light when the cell had been blue adapted (during PDA). The first response to an orange stimulus is small. The response recovers in the subsequent pulses of orange light until its recovery is complete by the fourth pulse. Trace (iv) shows the response to orange light if the cell had been orange-adapted. (Same as trace (i)). Note that the response pattern shown in trace (iv) is very different from trace (iii). These two traces serve as a control for Fig. 3b and c. b, The introduction of N_2 blocked the light-induced response. The blue pulses induced no measurable response while the animal was in the anoxic state. After the animal had recovered, its responses to the orange stimuli showed the same pattern as in the control (Fig. 3a (iii)) indicating that the blue stimuli were effective in inducing the PDA effect during anoxia. Intracellular recordings show that the membrane was in a high conductance state (the PDA effect) when the cell first recovered from anoxia. This shows that the induction of PDA is just as effective during anoxia as in the normal state. c, We stimulated the eye with five blue pulses and then three orange pulses while the animal was in an anoxic state. There was no observable response to any of these stimuli. After recovery, the response to orange light showed that the eye was in the orange-adapted state (see Fig. 3a (iv)). Since the amounts of blue light and orange light given in the anoxic state were the same as those used in the control (Fig. 3a), the results indicate that the induction of PDA and the reversal of this process were just as effective during anoxia as in the normal state.

from anoxia. In Fig. 3c we show the responses from an animal which was in the state of anoxia when the blue stimuli and the three subsequent orange stimuli were applied. None of these stimuli induced any observable response. The next few orange stimuli were applied after the animal had recovered from anoxia. As in the case of the control experiment (Fig. 3a(iii) and (iv)) the responses to these orange stimuli show a full recovery from the PDA effect induced by the blue stimuli. The results shown in Fig. 3 indicate that the induction of PDA and its reversal by orange light are just as effective during anoxia as in the normal state, even though there is no observable photo-response during anoxia. Experiments similar to the above have also been carried out using lowered temperature (on *Drosophila*) or metabolic poisons (on *Balanus* by R. Clark Lantz, A.M. and F.W.) as blocking agents of photo-responses. Results from these experiments are consistent with those shown in Fig. 3.

Since the spectral transition of the pigments and the induction of the PDA effect all took place during anoxia when no response was observed, the observation that the conductance increase associated with the PDA appears after the animal has recovered from anoxia indicates that the shifting of rhodopsin to metarhodopsin does not increase the conductance directly. There must be intermediate steps "tightly coupled" to the shifting of the pigments, or to the pigment states themselves which lead to the change in membrane conductance. Our results show that one or more of the early steps initiated by the shifting of the pigments is not sensitive to anoxia. Once initiated, it persists through the period of anoxia and expresses its effect on recovery. One or more of the later steps in the chain of events leading to a conductance increase is, however, sensitive to anoxia. But our results do not rule out the possibility that the anoxia-insensitive steps could be the metarhodopsin state itself.

The above results have been obtained by studying the PDA phenomenon. Several lines of evidence suggest that the mechanism of PDA generation closely resembles that involved in the generation of the light-coincident receptor potential^{17,18}. Thus, the conclusions presented here may apply also to the light-coincident transduction process.

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Blood pressure in snakes from different habitats

SNAKES seem to have evolved from burrowing lizards and are experiencing an impressive adaptive radiation. Terrestrial forms represent the probable ancestral type which gave rise to several lines of divergent radiation into aquatic and arboreal habitats^{1,2}. These shifts in habitat may have affected blood pressure regulation in snakes which, because of their shape, are inordinately subject to the hydrostatic effects of gravity. By acquiring aquatic habits, some species have eliminated most of the hydrostatic stresses on the cardiovascular system; conversely, arboreal species are potentially exposed to serious problems of blood flow and perfusion pressure. Our study indicates that adaptive evolutionary changes in arterial blood pressure regulation occurred during the transitions from terrestrial to aquatic or arboreal life in snakes. There are distinct trends towards low arterial pressure, with reduced regulatory ability in aquatic species and towards high pressure, with good homeostasis in an arboreal species.

To examine blood pressure and the extent of its control, we measured arterial pressure during tilting experiments involving nine species from aquatic, semi-aquatic, terrestrial and arboreal habitats (Table 1). Freshly captured animals were anaesthetised in ice and the caudal segment of the aorta occlusively catheterised with saline-filled, heparinised, polyethylene tubing. The animals were straightened out in appropriately sized Perspex tubes and allowed to recover from the surgery for at least 20 h before pressure measurements were taken. The temperatures of the snakes were maintained between 26 and 29 °C. Blood pressure was measured with Bell and Howell or Statham transducers, calibrated with water manometers and coupled to a Beckman Dynograph or Grass Polygraph. The transducers were placed at heart level and the tilt angle, head-heart distance, and total length of the snake in the tube were measured for calculations of pressure in the head and body centre. After each change in tilt angle, there was a large immediate change in pressure followed by a period of adjustment after which the pressure stabilised. The negligible kinetic energy of blood in snakes is ignored.

There is good correlation between habitat and both the absolute arterial pressure and the response to tilting. In the aquatic and semi-aquatic species, the trend is towards lower pressure (Table 1) as well as a decreased ability to compensate for the effects of postural change (Fig. 1). The opposite trend appears in the arboreal species. Phyletic comparison is especially good between the terrestrial elapids (*Notechis scutatus* and *Austrelaps superbus*) and their close relatives, the sea snakes (*Laticauda colubrina*, *L. semifasciata*, *Hydrophis ornatus*, and *H. belcheri*)^{3,4}. The trends are shown independently by the arboreal snake, *Boiga dendrophila*, and the semi-aquatic snake, *Cerberus rhynchops*, which are both venomous rear-fanged colubrids. Only the non-venomous *Chersydrus granulatus* lacks a taxonomically close relative within the study. Like the other aquatic snakes, however, it shows relatively low blood pressure and

Table 1 Mean arterial blood pressure and its regulation in various snakes

Species (n)	Habitat	Mean b.p.* (mmHg)	Weight (g)	Total length (cm)	Head-Heart distance† (%)	Slope‡
<i>Boiga dendrophila</i> (3)	Arboreal	74	185	126	17	+0.25
<i>Austrelaps superbus</i> § (2)	Terrestrial	61	119	66	17	+0.01
<i>Notechis scutatus</i> (9)	Terrestrial	49	282	93	16	+0.14
<i>Cerberus rhynchops</i> (3)	Semi-aquatic	35	128	64	24	+0.05
<i>Laticauda colubrina</i> (3)	Semi-aquatic	38	495	116	30	+0.07
<i>L. semifasciata</i> (3)	Semi-aquatic	32	431	110	20	+0.02
<i>Chersydrus granulatus</i> ¶ (2)	Aquatic	27	239	77	43	+0.03
<i>Hydrophis belcheri</i> (2)	Aquatic	33	418	92	38	+0.23
<i>H. ornatus</i> (3)	Aquatic	22	512	97	34	+0.10

*Horizontal

†Head-heart distance $\times 100/\text{total length}$.

‡Slope of regression line fitted to head-up tilting data (Fig. 1).

§Formerly *Denisonia superba*.¶Formerly *Acrochordus granulatus*.

reduced control. The semi-aquatic and aquatic species represent three, or possibly four, independent shifts from land to water, each shift apparently accompanied by a consistent change in blood pressure regulation.

In Fig. 1 we show blood pressure at the centre of the snake, where there should be no pressure change because of tilting alone. If pressure remains constant at this point during tilting, then the snake is acting as a simple fluid-filled tube and is sustaining, in its upper half, a drop in pressure equal to the change in the hydrostatic blood column. Should the pressure at the centre of the snake increase with tilting, then there is physiological adjustment to augment pressure in the upper half; a fall in central pressure means that the pressure in the upper half falls excessively, probably because cardiac output is reduced by venous pooling in the lower half. Table 1 gives the slopes of regression lines fitted to the data for head-up tilting. *B. dendrophila* and *N. scutatus* are the best regulators and the two *Hydrophis* species are the poorest.

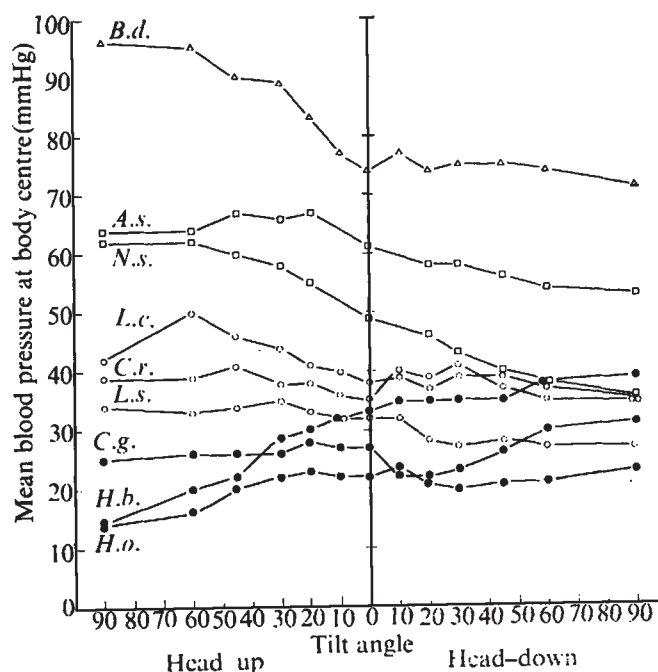


Fig. 1 Mean arterial blood pressure at the body centre in snakes as a function of tilt angle. Values are stable mean pressures following the initial adjustment (see text). Initials designate the species which are listed in Table 1. Symbols divide the species according to habitat: arboreal (Δ), terrestrial ($\square$), semi-aquatic ($\circ$), and aquatic ($\bullet$).

The physiological adjustments responsible for regulation of blood pressure are complex and are beyond the scope of this paper but our data indicate that regulation is achieved through changes in both heart rate and vasomotor tone (H.B.L. and R.S.S., unpublished). As in lizards⁵, vasomotor tone seems to be the most important factor in snakes.

The loss of regulatory control in aquatic snakes is probably attributable to the equalising effect of external hydrostatic pressure. Because totally aquatic snakes are never subjected to significant changes in transmural pressure, the need for regulation of venous tone does not exist. Alternatively, there should be strong selective advantage for vasomotor reflexes in snakes which move in three dimensions out of water. It is perplexing that, when measuring interstitial fluid pressure in large snakes during tilting, Scholander and coworkers⁶ found better homeostasis in the aquatic anaconda than in the arboreal boa constrictor. They did not measure blood pressure, however, and the pressure relationships in snakes between blood and interstitial fluid are unknown.

A possible adaptive value for higher absolute pressure in terrestrial and arboreal snakes is one of minimising the effects of postural changes on perfusion. As a simplified hypothetical example, assume that two similarly sized snakes having different blood pressures are tilted and both maintain constant central pressure. Because arterial pressure in the head is a function of vertical distance between the body centre and the head, both snakes experience the same absolute pressure decrease in the head, but the one with the higher central pressure shows a lesser proportionate change in head perfusion. It seems to be important for snakes to maintain adequate perfusion pressure in the brain, some snakes 'faint' if postural changes prevent sufficient blood flow to the head and reptiles generally do not tolerate stagnant anoxia (lack of oxygen resulting from stopped circulation) in the brain⁷. Blood pressure in the heads of the two *Hydrophis* species became negative at tilting angles of 45° or greater. At 90°, both *Laticauda* species and *C. granulatus* exhibited negligible head blood pressure.

In relation to the importance of maintaining pressure in the brain, it is interesting that the heart is proportionately closer to the head in terrestrial and arboreal snakes than in aquatic ones (Table 1). There is thus a trend in terrestrial snakes towards limiting the length of the vertical blood column to that which the heart can support. In aquatic species, on the other hand, the trend is towards a midbody position of the heart. This tends to equalise the flows and pressures required to perfuse the anterior and posterior portions of the animal.

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Effect of plant cytokinins on microfilaments and tight junction permeability

IN epithelia classified as “leaky”, net transport efficiency of NaCl and water may be determined not only by cellular metabolic activity, but also by tight junction permeability and the conjugate physical driving forces acting between lumen and intercellular space. The significance of paracellular ion movement across leaky epithelia would become more apparent if a cytoplasmic mechanism for regulation of tight junctional permeability could be defined. The structure-function observations reported here suggest that the microfilament system may have such a function. Rapid and reversible increases in shunt path electrical resistance were observed in *Necturus* gallbladder exposed to plant cytokinins. In electron micrographs zeiotic transformation of the luminal membrane was associated with

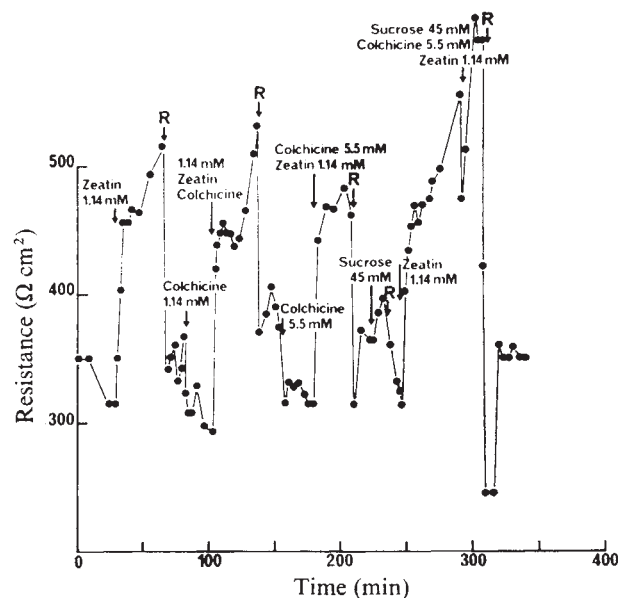


Fig. 1 An experiment illustrating resistance response to zeatin (1.14 mM in the mucosal bath). Transepithelial resistance is given on the ordinate. The initial increase in resistance occurs within 5 min and is completely reversible when zeatin is replaced by Ringer solution (R) containing the same concentration of DMSO. In this experiment, colchicine caused a decrease in resistance and did not inhibit the zeatin response.



Fig. 2 Zeiosis following a 30-min mucosal exposure to kinetin (1.0 mM). Bizarre, enlarged microvillae demonstrate disrupted, clumped and swollen microfilaments. ($\times 24,500$).

disruption of microfilaments and in freeze fracture, with reorientation and disorder of tight junctional elements.

Gallbladders were slightly stretched over a 0.35 cm² opening in a Plexiglas chamber, mucosal side up¹; experiments were carried out at room temperature. The composition of the standard Ringer bathing solution was (in mM l⁻¹) NaCl 95, NaHCO₃ 13, CaCl₂ 1.8, MgCl₂ 1.0, KCl 4.5, KPO₄ 0.7, glucose 2.0 (220 mosmol l⁻¹), pH 7.4. Polyvinylpyrrolidone (molecular weight, 40,000, 20 g l⁻¹), was added to the serosal solution. Transepithelial potential difference (PD_{tr}) and resistance (R) were measured, the latter by a four-electrode technique. Relative cell membrane resistances were measured by the voltage divider ratio through an intracellular microelectrode.

Plant cytokinins are purine derivatives best known for their ability to induce plant cell division. Kinetin, zeatin, or zeatin riboside were dissolved in dimethyl sulphoxide (DMSO) and then in the Ringer's solution (final concentration of DMSO was no more than 0.5%). R and PD_{tr} were evaluated for mucosal cytokinin concentrations of 0.1–2.0 mM. Bladders were fixed *in situ* with 1 or 2% glutaraldehyde in 0.05 M phosphate buffer, pH 7.0. Freeze fracture was done at -150°C on 20% glycerol-impregnated tissue, previously fixed in glutaraldehyde.

In the control state, transepithelial electrical parameters were similar to those reported by others^{1–3}. PD_{tr} ranged from 0 to -6 mV, mean -1.74 mV ± 0.45 (s.e.) ($n = 12$), mucosal side

negative. The resistance averaged $236 \Omega \text{ cm}^{-2} \pm 26$ (s.e.) ($n = 11$). Dilution potentials, achieved by isosmotic substitution of 45 mM sucrose for NaCl, yielded a $9.9 \text{ mV} \pm 0.6$ (s.e.) ($n = 8$) shift in PD_{tr} , mucosa positive, indicating that the shunt pathway is cation selective. Cell PD_{tr} ranged from -39 to -66 mV , mean $-44.1 \text{ mV} \pm 0.7$ (s.e.) ($n = 27$).

Addition of cytokinins to the mucosal compartment did not change PD_{tr} , cellular PD or dilution potentials. Transepithelial resistance increased rapidly during the first 1–5 min reaching a peak value in 20–30 min and was rapidly reversible (Fig. 1). Cytokinin added to the serosal bath alone caused a delayed increase in resistance, but did not add to the mucosal response. The increase in resistance was roughly linearly dependent on mucosal concentration. Resistance doubled with mucosal bath concentrations of 2.0 mM zeatin or kinetin. The voltage divider ratio (R_{mucosa}/R_{serosa}) in four bladders decreased from 1.25 ± 0.13 to 0.79 ± 0.04 (s.e.) ($n = 23$) because of a decrease in R_{mucosa} . All changes were reversible after the cytokinin was washed from the mucosal compartment. Kinetin, the least soluble, gave the most gradual and persistent response. The apparent decrease in luminal membrane resistance induced by cytokinins may result from increased surface and/or a nonspecific distension of the luminal membrane, an interpretation suggested by the ultrastructure. Focal evagination of the luminal membrane (zeiosis)⁴ associated with disruption of attached microfilaments (Fig. 2) was observed as early as 5 min after cytokinin exposure, initially appearing at distal extensions of microvillae located at the apogee of the luminal membrane.

The concomitant increase in transepithelial resistance can safely be ascribed to an increase in shunt (tight junction plus intercellular space) resistance, previously reported to be 12–26 times smaller^{1,3} than R_{serosa} plus R_{mucosa} . More explicitly, current passage must be impeded at the tight junction, since no morphological changes were observed in the intercellular space and R_{mucosa} fell after cytokinin treatment.

In tissue exposed to cytokinins, desmosomes seemed more

Fig. 3 Freeze fracture (EF face) in control gallbladder exposed to Ringer with 0.5% DMSO. The furrows are regularly spaced and horizontally oriented. The most abluminal element (arrow) is continuous with rare basal extensions ($\times 59,100$).

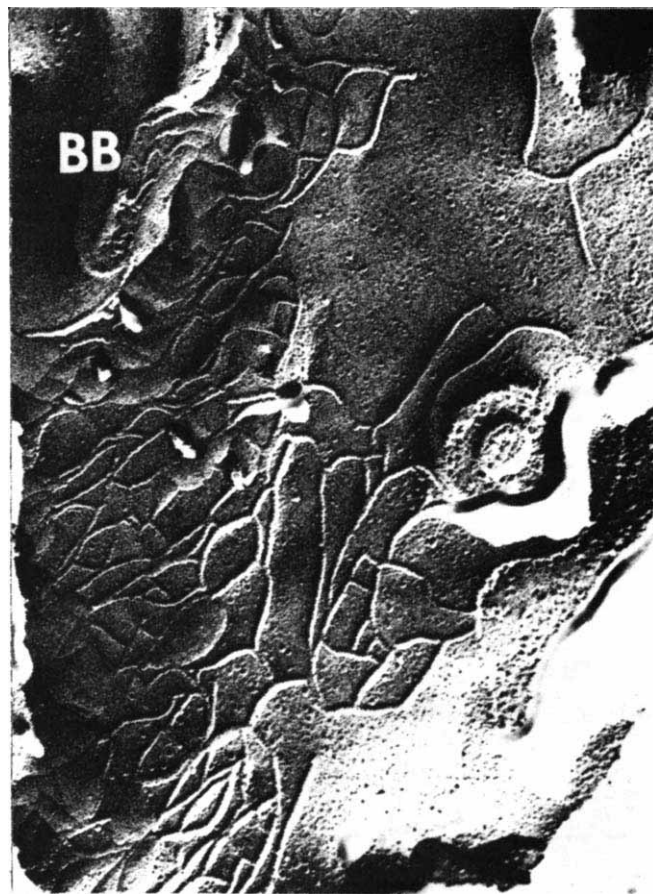


Fig. 4 Freeze fracture (PF face) 1 h after exposure to 1.0 mM zeatin. The brush border on the left is labelled (BB). Ridges are disorganised and oriented more perpendicular to the brush border than in control. The most abluminal ridge is discontinuous and open ends are often randomly directed down the lateral cell membrane ($\times 59,100$).

numerous and were frequently asymmetrical with short tonofibrils. With freeze fracturing, clusters of intramembranous particles on EF and PF fracture faces were found. All of these features are characteristic of desmosome assembly⁵. In electron-micrographs "fusion" lines between cells comprising the zona occludens (tight junction) showed focal areas of slight separation. The zona occludens seemed accentuated by clumping of microfilaments in the adjacent cytoplasm, whereas in control sections, most microfilaments were in apposition to the zona adherens and not the zona occludens.

On freeze fracture (Fig. 4), the furrows and ridges characteristic of tight junctions were not arranged in the regular configuration seen in control bladders (Fig. 3). Junctional strands seemed to change orientation from parallel to perpendicular to the luminal border and single open ended strands extended deep in the basal direction. The network was both more extensive and more disordered; in some areas, parallel ridges abutted and in other areas were widely separated. At basal extensions of the junction, new strand formation was suggested by beading and membrane indentation. These findings are similar to that reported in rat liver exposed to phalloidin, a drug also associated with ultrastructure changes in microfilaments⁶.

We postulate that control of paracellular route of ion permeation, dominated by tight junction, involves specific interaction between microfilaments and the specialised junctional domains of plasma membrane. Since the electrical and structural changes occur within a short period after mucosal exposure to cytokinins and are reversible, it is unlikely the new protein synthesis is required, and therefore, the observed alteration in

junctional reorganisation is due to re-use of pre-existing junctional components. The exact molecular mechanism, possible intermediary steps, and the modifying role of cytokinin action between the microfilament system and junctional assembly are not yet elucidated. These studies do, however, indicate that microfilaments are intimately associated with development and function of a type of plasma membrane specialisation, the tight junction (and possibly the desmosome). Most importantly, these data could lead to an understanding of the physiological control of tight junction permeability and its relationship to epithelial transport.

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Evidence for a dual role for oxygen in control of abscission

ABSCISSION—the shedding of leaves, fruits and other plant parts—is important in the morphogenesis and homeostasis of plants, and valuable agricultural practices are based on its control. Carns *et al.*¹ demonstrated that oxygen is essential for abscission; they found that less than 21% O₂ retarded abscission while more than 21% and up to 40% O₂ accelerated abscission; concentrations above 40% gave a uniform maximum rate of abscission (inset of Fig. 1). Acceleration of abscission by O₂ was confirmed in two investigations^{2,3}, but Abeles and Gahagan⁴, who studied abscission responses to O₂ in the presence of 0.01 p.p.m. ethylene, observed no acceleration with O₂ concentrations above 21%. They suggested that the accelerations observed before were caused by ethylene, which sometimes contaminates commercial O₂. We have re-examined the abscission responses to O₂ in carefully monitored conditions and found an unusual double sigmoid relationship between abscission and oxygen concentration.

Explants of cotton (excised cotyledonary nodes of *Gossypium hirsutum* L. cultivar Acala SJ-1) were held at 30 °C in small chambers with a continuous gas flow. O₂, CO₂ and ethylene were monitored by gas chromatography. Twenty explants (40 abscission zones) were placed in each chamber; percentage abscission was determined after 48 h and plotted in Fig. 1. The flow system permitted an average fluctuation of $\pm 2\%$ in O₂ concentration, and explants varied about $\pm 10\%$ in their rates of abscission. Consequently, both variations were taken into account in fitting the curve to the points (see legend).

Figure 1 shows that abscission was retarded below approximately 10% O₂. With 10–20% O₂, abscission rates equalled control (air) rates. Above about 25% O₂, abscission was accelerated strongly.

We attribute the retardation of abscission by concentrations of O₂ below 21% to restriction of O₂ to respiratory processes. Abscission depends on the synthesis of pectinases

and/or cellulases⁵, which in turn depend on ATP. Carns⁶ found that respiratory inhibitors also inhibited abscission. Abeles and Gahagan⁴ found that the rate of abscission paralleled the rates of respiration (as measured by oxygen uptake). We suggest that the acceleration of abscission by more than 21% O₂ is due to a second O₂-requiring process that promotes abscission. The double sigmoid shape of the curve indicates that two distinct oxidative processes are involved.

Measurements of ethylene and CO₂ evolution from the explants gave some insight into the nature of the acceleration of abscission by high concentrations of O₂. Ethylene production of the explants with 5–40% O₂ was essentially independent of O₂ concentration (ethylene was determined with a relative accuracy of about 2 p.p.b.). These data ruled out the possibility that the acceleration resulted from an increased production of ethylene due to the influence of O₂. The CO₂ output did not increase appreciably in high concentrations of O₂, suggesting that the acceleration of abscission was not due to general stimulation of respiratory processes. This is in accordance with the generally accepted view that O₂ does not limit respiration in concentrations above 21%, or even above the extinction point in some cases⁷. (Data for ethylene and CO₂ are being published elsewhere.) In a separate experiment abscission rate was measured in nine chambers receiving high concentrations of O₂ containing vials of mercuric perchlorate to absorb ethylene. There was strong acceleration of abscission in each chamber although ethylene never exceeded 3 p.p.b., well below the level that can influence abscission. Thus, contamination of O₂ by ethylene was not a significant factor in our experiments.

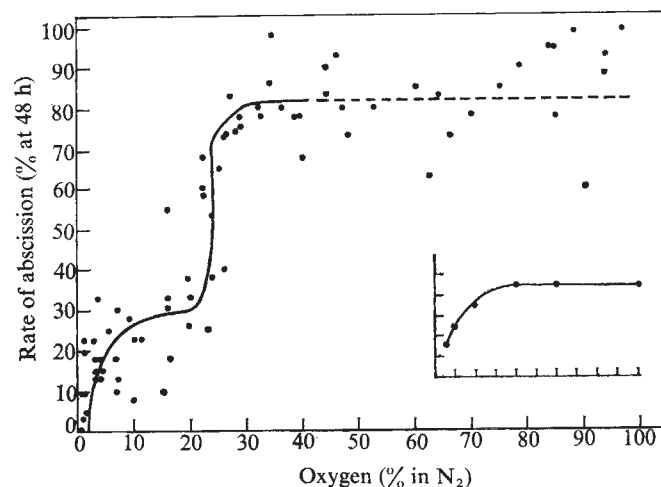


Fig. 1 Rate of abscission of cotton explants as influenced by oxygen concentration. There are 78 data points from experiments conducted in five different weeks with each point representing the percentage abscission of 40 abscission zones. Details of the operation of the flow system are given in ref. 9. Points from 0 to 40% O₂ were subjected to a detailed statistical analysis, taking into account errors in both abscission and O₂. Orthogonal regression¹⁰ on the function

$$f(x) = a + \frac{b}{(x+1)} + c \tanh[d(x-e)]$$

with unit weights and a constant scale factor of 5/7 applied to the y variable yielded the double sigmoid curve shown (solid line). The above function was chosen for its ability to assume either a smooth form or a double sigmoid form. Abscission is apparently independent of oxygen concentration in the 40–100% range (dashed line). The final variables and standard error of the best fit are: $a = 58.16$; $b = -80.20$; $c = 23.54$; $d = 10.01$; $e = 24.78$; $s.e. = 3.92$. Normalisation using air controls, and high O₂ concentration controls resulted in similar double sigmoid curves. (Details of the statistical analysis will be presented elsewhere.) The inset shows the effect of oxygen concentration (x axis) on the abscission rate (y axis) of bean explants held in sealed containers. Each point is an average of the response from 20 to 90 abscission zones (after Carns *et al.*¹).

There are several possible reasons why Abeles and Gahagan⁴ did not observe acceleration of abscission by high concentrations of O₂. First, the presence of 0.01 p.p.m. ethylene in their experiments would have impaired their ability to detect acceleration. Second, their measurements of abscission were made only 4 h after explants were exposed to the experimental atmospheres, and it is possible that high concentrations of O₂ are effective only after a longer time. Third, acceleration of abscission by high concentrations of O₂ may be due to interference with the insensitivity to ethylene that commonly follows explant excision. If the acceleration we report does represent a response to this ethylene, Abeles and Gahagan could not have observed it, for 19 h elapsed between explant excision and exposure to O₂ in their experiments. Finally, the ability of O₂ to accelerate abscission may vary from species to species and even from variety to variety.

Ethylene could still be indirectly related to this acceleration through an effect of O₂ on tissue sensitivity to ethylene, particularly during the first 12 h after excision. Another possible reason for the effect of high concentrations of O₂ is oxidative inactivation of indolyl acetic acid (auxin) catalysed by the enzyme complex known as "IAA-oxidase". Its proposed free radical mechanism⁸ could be responsive to high concentrations of O₂. Increased IAA-oxidase activity and decreased concentrations of auxin are known to be correlated with abscission⁹. It is also possible that some other process that requires a high concentration of O₂ accelerates abscission.

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Photosynthetic regulatory protein found in animal and bacterial cells

WE recently reported that two proteins, assimilation regulatory protein a (ARP_a) and assimilation regulatory protein b (ARP_b), are needed for the light-induced activation of certain enzymes of photosynthetic CO₂ assimilation^{1,2}. ARP_a and ARP_b link the strong reducing power of ferredoxin, reduced photochemically, to the control of key enzymes through a mechanism that, until now, was considered to be unique to chloroplasts^{3,4}. In a search to determine the distribution of this type of control mechanism, we have observed that the more thoroughly characterised of these proteins, ARP_b, occurs widely in living cells. The results provide evidence that protein modification reactions of the ferredoxin-linked type described for chloroplasts occur in non-photosynthetic as well as photosynthetic organisms.

ARP_b from different sources was assayed by its capacity to activate homogeneous preparations of spinach chloroplast fructose 1,6-bis-phosphatase^{4,5} in a system in which the chloroplast ferredoxin and ARP_a components were replaced by the non-physiological sulphhydryl reagent dithiothreitol^{2,4,5}. In this assay, the inorganic phosphate cleaved from the C₁ position of fructose 1,6-bis-phosphate

Table 1 Occurrence of ARP_b in different types of photosynthetic organisms

	ARP _b (units per mg protein)
Green plants	
Spinach chloroplasts	3.3
Sugar beet chloroplasts	8.1
Blue-green algae	
<i>Nostoc muscorum</i>	0.6
Photosynthetic bacteria	
<i>Chlorobium thiosulfatophilum</i>	6.2
<i>Chromatium vinosum</i>	1.5
<i>Rhodospirillum rubrum</i>	1.1

The soluble protein fraction from isolated spinach chloroplasts (chloroplast extract) and cell-free extracts of the different micro-organisms were prepared in a solution containing 25 mM Tris, pH 8.0, and 1.4 mM 2-mercaptoethanol, as described previously^{3,4,6}, and adjusted to pH 4.5 with 2 N formic acid. The precipitate was centrifuged off (5 min, 48,200g); the supernatant fraction was adjusted to pH 7 with 1 N ammonium hydroxide and dialysed overnight at 4 °C against the preparative solution. For assaying ARP_b, the reaction mixture contained the indicated dialysed pH 4.5 supernatant fraction, fructose 1,6-bis-phosphatase, 8 µg, and the following (in µmol to a final volume of 0.5 ml): Tris-HCl buffer (pH 7.9), 50; MgCl₂, 0.5; dithiothreitol, 2.5; and fructose 1,6-bis-phosphate, 3. The reaction was started by adding fructose 1,6-bis-phosphate and was continued for 30 min at 25 °C. The reaction was stopped by adding 0.5 ml of 10% trichloroacetic acid and, after centrifuging off the precipitate, inorganic phosphate was estimated by a modified Fiske-SubbaRow procedure⁴. Protein was estimated by a modified phenol reagent procedure⁴.

by the phosphatase enzyme is measured colorimetrically. The assay gave reproducible results with ARP_b in cell-free preparations from which the native fructose 1,6-bis-phosphatase had been largely or totally removed by precipitation at pH 4.5 and dialysis of the resulting supernatant fraction overnight at alkaline pH (refs 4 and 5). One unit of ARP_b is defined as the ARP_b-mediated release of 1 µmol of inorganic phosphate by 8 µg of fructose 1,6-bis-phosphatase enzyme in the conditions in Table 1.

Table 1 shows that ARP_b was present in comparable amounts in dialysed pH 4.5 supernatant fractions obtained from the major types of photosynthetic organisms: higher plants (chloroplasts of spinach and sugar beet), algae (*Nostoc muscorum*), and each of the three major types of photosynthetic bacteria (green sulphur, *Chlorobium thiosulfatophilum*; purple sulphur, *Chromatium vinosum*, and purple non-sulphur, *Rhodospirillum rubrum*). Such a distribution of ARP_b in photosynthetic cells prompted us to examine non-photosynthetic counterparts for this protein. This search led to the finding of ARP_b not only in non-photosynthetic plant tissues (spinach seeds and roots) but also in heterotrophic bacteria—organisms which obtain energy by the breakdown of organic compounds and which cannot utilise radiant energy (Table 2). ARP_b was found in heterotrophic bacteria of both the aerobic (*Escherichia*

Table 2 Demonstration of ARP_b in non-photosynthetic plant tissues and heterotrophic bacteria

	ARP _b (units per mg protein)
Plant tissues	
Spinach seeds	3.1
Spinach roots	1.6
Aerobic bacteria	
<i>Escherichia coli</i>	4.9
<i>Bacillus subtilis</i>	1.6
Anaerobic bacteria	
<i>Clostridium pasteurianum</i>	1.3
<i>Desulfovibrio desulfuricans</i>	2.0

Cell-free extracts of seeds and roots were prepared by blending tissues and centrifuging the resulting slurry as in Table 1. The ratio of tissue to buffer for blending was 1:2 (weight: volume). Before disruption, seeds were soaked for 8 h in flowing tap water. Other conditions were as in Table 1.

Table 3 Effect of light on the development of ARP_b in barley seedlings

	ARP _b (units per mg protein)
Seedlings germinated in darkness	9.6
Seedlings germinated in light	7.5

Barley seedlings were germinated from seeds placed in vermiculite saturated with water. The pH 4.5 supernatant fraction was prepared from seedlings germinated for 7 d at 25 °C and assayed as in Table 2.

coli, *Bacillus subtilis*) and anaerobic (*Clostridium pasteurianum*, *Desulfovibrio desulfuricans*) types. Additional evidence that the formation of ARP_b is independent of light came from the finding that light- and dark-germinated barley seedlings contained similar amounts of ARP_b (Table 3).

The occurrence of ARP_b in non-photosynthetic plant and bacterial cells raised the possibility that this protein might also be present in tissues of animal origin. Analyses were therefore conducted with dialysed pH 4.5 supernatant fractions from rabbit liver—a source rich in enzymes and

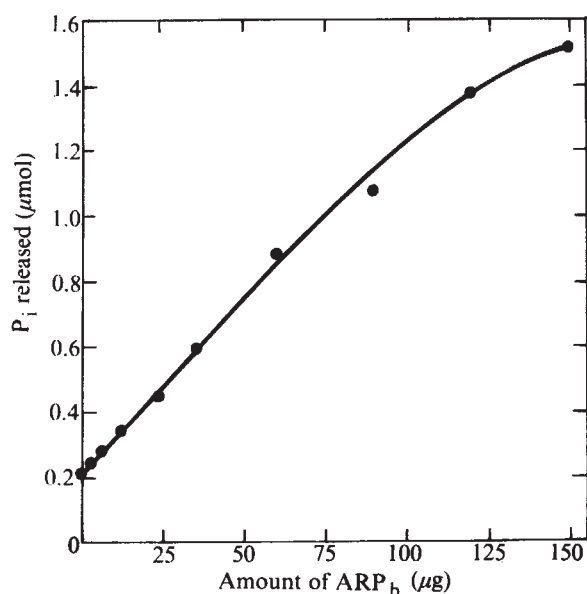


Fig. 1 The indicated amounts of a highly purified liver ARP_b preparation were assayed as described in Table 1. P_i corresponds to inorganic phosphate.

associated regulatory proteins. The initial results, while indicating the presence of ARP_b, revealed that in this case the preparation required additional purification to eliminate interference in the assay by the native fructose 1,6-bisphosphatase. Accordingly, the pH 4.5 supernatant fraction of liver cells was further fractionated by a procedure consisting of precipitation with ammonium sulphate and successive chromatography on Sephadex G-100, diethylaminoethyl (DEAE) cellulose and hydroxyapatite columns. The purified preparation so obtained consistently showed ARP_b activity (Fig. 1). Other properties of the purified liver ARP_b preparation will be published elsewhere.

In summary, the present findings show that ARP_b, a protein first described for chloroplasts, is widely distributed in nature. ARP_b was found in photosynthetic and non-photosynthetic plant cells, photosynthetic bacteria, anaerobic and aerobic heterotrophic bacteria, and animal cells. This distribution of ARP_b extends to the major types of living cells the possibility of a mechanism of protein modification of the reductive type that in chloroplasts is mediated by reduced ferredoxin. Those cells which lack a ferredoxin of the plant or bacterial type^{7,8} accordingly would utilise other reductants to this end. The identity of

the reductant used by such cells in lieu of ferredoxin is unknown.

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Native collagen is not a substrate for the collagen glucosyltransferase of platelets

The adhesion of platelets to collagen in the subendothelial tissue is generally considered to be the primary step in haemostasis following vascular injury. The biochemical basis of this interaction is not fully understood but it has been suggested by Jamieson and coworkers^{1,2} that the adhesion of platelets to collagen is mediated by the formation of an enzyme-acceptor complex between a glucosyltransferase on the platelet membrane and incomplete carbohydrate residues present in collagen. We report here studies confirming the presence of collagen glucosyltransferase activity associated with platelets but investigations of the influence of collagen substrate conformation on enzyme activity suggest that the above hypothesis is probably untenable.

The enzyme assays used to study collagen glucosyltransferase activities associated with human platelets^{3–5} were originally used in studies claiming to show collagen glucosyl- and galactosyltransferase activities in guinea pig skin^{6,7}. These procedures have been criticised for their lack of specificity^{8,9} and methods designed to measure specifically the synthesis of hydroxylysine-¹⁴C-glycosides have subsequently been described^{8–11}. In this study, a preliminary characterisation of the collagen glucosyltransferase of porcine platelets has been carried out using the assay procedures of Harwood *et al.*¹¹. Platelets were prepared by the method of Lopaciuk and Solum¹² from blood anticoagulated with 5.4 mM EDTA. The preparations were sonicated

Table 1 Comparison of bivalent cations as cofactors for collagen glucosyltransferase

Cation added	Concentration (mM)	Glucosyltransferase activity (d.p.m.)	relative activity (%)
None		2,825	3
Mn ²⁺	10	91,850	100
	20	88,510	96
Mg ²⁺	10	31,218	34
	20	26,006	28
Co ²⁺	10	14,437	16
	20	15,240	17
Ca ²⁺	10	9,721	11
	20	8,196	9

The reaction using glucose-free corneal gelatin as substrate was carried out as described in Fig. 1 except that the metal cofactor was varied.

(2×5 s) at 4°C in 0.02 M Tris-HCl buffer, pH 7.4, containing 0.15 M NaCl and $10\ \mu\text{M}$ dithiothreitol, dialysed against this buffer (200 volumes) and then assayed for collagen glucosyltransferase activity. The substrate employed was glucose-free bovine corneal gelatin which contained 11 hydroxylysine residues per 1,000 amino acids of which approximately seven residues were substituted with galactose. A linear relationship between enzyme concentration and the synthesis of ^{14}C -glucosylgalactosyl-hydroxylysine was obtained. Varying the substrate concentration gave Michaelis-Menten kinetics for the reaction and the K_m for glucose-free corneal gelatin was $5.35\ \text{g l}^{-1}$ (Fig. 1). Studies on the effect of pH on enzyme activity showed an optimum between pH 6.8 and 7.4, which differs markedly from the pH optimum of 5.7 initially determined for this enzyme in human platelets using a collagenous substrate³.

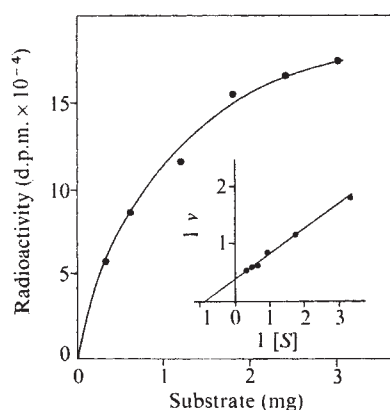


Fig. 1 Effect of the concentration of corneal gelatin on the glucosyltransferase activity of porcine platelets. Collagen was prepared and purified from bovine corneal tissue using pepsin solubilisation and salt precipitation techniques and then treated with 0.025 M H_2SO_4 for 20 h at 100°C to remove selectively glucose residues. After hydrolysis the sample was neutralised with 2.0 M NaOH, and varying amounts of this substrate were used directly in the assay. The incubation was carried out for 90 min at 37°C in a total volume of 200 μl which also contained 15 μmol of Tris-HCl buffer, pH 7.4, 2 μmol MnCl_2 , 0.4 μmol of 2-mercaptoethanol, 10 μl of 0.1% Triton X-100, 0.67 μCi of UDP- ^{14}C glucose, and 0.8 mg of platelet enzyme protein. Precipitation of the protein with acetone and subsequent determination of the ^{14}C -glucosylgalactosyl-hydroxylysine synthesised was achieved by alkaline hydrolysis, desalting and paper electrophoresis of the samples as described in ref. 11. In the double-reciprocal plot the values shown in the abscissa are reciprocal values of substrate concentration in mg per 200 μl , and the values shown in the ordinate are reciprocal values of the velocity in $10^{-5} \times$ radioactivity of the product formed in 90 min.

The effect of varied Mn^{2+} concentration on glucosyltransferase activity was examined and the optimum Mn^{2+} concentration found to be 10 mM. Experiments to determine whether other bivalent metals could replace the manganese indicated that Mg^{2+} , Ca^{2+} and Co^{2+} were much less effective cofactors (Table 1). These results are similar to those reported for collagen glucosyltransferase from human platelets³, rat renal cortex⁸ and embryonic chick tissues^{10,13}. A further similarity to the human platelet³ and chick embryo enzyme¹³ is the susceptibility of the porcine platelet enzyme to marked inhibition by *p*-mercuribenzoate and *N*-ethyl maleimide: 50% inhibition occurred with both reagents at about 0.3 mM. These observations and the finding that the enzyme activity was also stimulated approximately 30% above controls by 2×10^{-4} M 2-mercaptoethanol suggest that free sulphhydryl groups are present in the active centre of the enzyme. Since platelet adhesion to collagen is inhibited by sulphhydryl inhibitors¹⁴, the above data can be

quoted in support of the collagen glucosyltransferase theory of collagen-platelet adhesion.

According to the hypothesis, however, the first step must be the interaction of the platelet enzyme with galactosyl-hydroxylysine residues in collagen. The use of hydroxylysine-galactose as substrate for the human platelet enzyme has been reported briefly¹⁵ but observations with this substrate cannot necessarily be extrapolated to support the hypothesis that the natural substrate of the platelet enzyme is native collagen. We have therefore re-examined this question and the effect of substrate conformation on the glucosylation reaction promoted by the platelet enzyme was studied by comparing the efficiency of native corneal collagen and heat-denatured corneal collagen as substrates. Because the collagen triple helix is readily denatured above 38°C , these experiments were conducted at 30°C to ensure that the native corneal collagen retained its triple helical conformation. A marked difference was observed between the native and denatured collagen in that when the reaction was studied as a function of time and enzyme concentration (Fig. 2), no synthesis of ^{14}C -glucosylgalactosyl-hydroxylysine was observed with native collagen. These results are analogous to those reported for the collagen glucosyltransferase involved in the intracellular glucosylation of procollagen¹⁶ and suggest that the platelet enzyme also requires the collagen substrate to be non-helical.

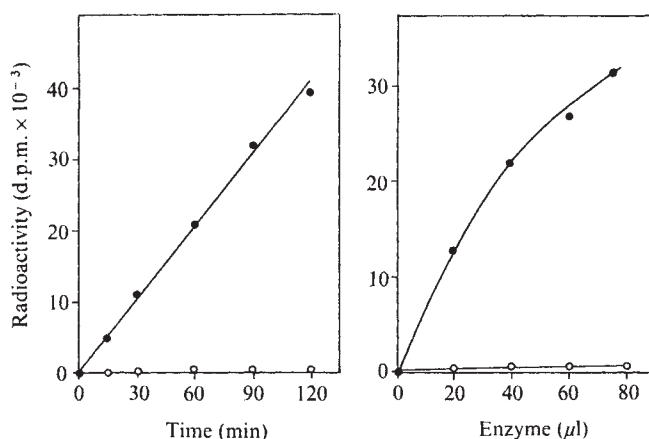


Fig. 2 Comparison of native and heat-denatured corneal collagen as substrates for collagen glucosyltransferase. The reaction was studied as a function of time and enzyme concentration and in both experiments the substrate concentration was $2\ \text{mg ml}^{-1}$. The native collagen used was prepared by pepsin digestion and purified under non-denaturing conditions. The optical rotation ($[\alpha]_D$) of this collagen was found to be -430° which indicated that the collagen was in the triple helical form. The heat-denatured collagen substrate was prepared immediately before use by placing a portion of the above preparation at 60°C for 10 min followed by rapid cooling in ice. The optical rotation of this denatured substrate was -135° . The reaction conditions were as described in Fig. 1, except that the incubation temperature was 30°C . $\circ$, Native (triple helical) substrate; $\bullet$, heat-denatured substrate.

Thus it seems that following vascular injury the adhesion of platelets to native collagen is unlikely to be mediated by the collagen glucosyltransferase. The roles of this enzyme and also collagen galactosyltransferase which we find to be present in porcine platelet preparations remain to be established.

Basic to the original hypothesis of Jamieson *et al.*^{1,2} were their observations that collagen glucosyltransferase activity was located primarily on the outer surface of the plasma membrane of human platelets³. We have undertaken a study of the location of collagen glucosyltransferase in subcellular fractions of porcine platelets isolated by the method

of Barber and Jamieson¹⁷. Using the specific enzyme assay we find that considerable activity occurs in the cytosol fraction as well as in the membrane and of particular interest is the presence of high amounts of collagen glucosyltransferase activity in freshly prepared platelet-free plasma; further details will be published later. The characterisation of the plasma enzyme and its relationship to the platelet enzyme are under investigation, but these observations must cast further doubts on the role of collagen glucosyltransferase in platelet-collagen adhesion.

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Changes in viral envelope structure preceding infection

THE first stage of infection by enveloped paramyxoviruses probably involves fusion of the viral envelope with the cell plasma membrane¹. During the course of an *in vitro* investigation into the mechanism of cell-cell fusion induced by Sendai virus it has become apparent that prior to the membrane fusion event the viral envelope undergoes a dramatic change in structure and this altered structure allows specific sites of fusion of viral envelopes with the plasma membrane to be identified in freeze fracture replicas.

Previous thin section studies have shown fusion of Sendai virus particles with the plasma membrane^{2,3} but these studies have not revealed any membrane specialisations which might occur. Freeze-fracture studies have shown a virus-induced aggregation of intramembranous particles during cell-cell fusion⁴ but have not shown actual fusion of viral envelopes with the plasma membrane.

In vitro, cell-cell fusion is carried out by first agglutinating a suspension of cells with Sendai virus at 4 °C. Fusion is a temperature-sensitive event, and occurs following a brief incubation at 37 °C (ref. 5). In these conditions fusion of viral envelopes with the cell membrane also occurs. In fact, cell-cell fusion probably requires an intermediate step involving fusion with the viral envelope⁶. In the present studies 1 ml of a 2% suspension of washed human erythrocytes (Type O) in Hank's Balanced Salt Solution⁷ (Hank's BSS) was agglutinated with 1 ml of

Sendai virus (1,000 haemagglutinating units ml⁻¹ for 1 h at 4 °C). Samples were then incubated for up to 1 h at 37 °C. Fusion was stopped by the addition of an equal volume of 6% glutaraldehyde and fixation continued for 30 min. Following infiltration with 25% glycerol cells were rapidly frozen in Freon 22 and fractured at -110 °C in a Denton freeze-fracture machine. Platinum carbon replicas were prepared for electron microscopy.

Freeze-fractured Sendai virus particles reveal internal aspects of their envelope structure. Fractures produce two complementary split membrane faces: P faces which belong to the cytoplasmic half of the membrane and face the exterior and E faces which belong to the external half of the membrane and face the cytoplasm⁸. At 4 °C, concave E fracture faces have numerous ~14-nm diameter intramembranous particles (Fig. 1a) while convex P faces have a complementary arrangement of pits (Fig. 1b). Following a brief incubation at 37 °C some virus particles undergo a dramatic change in the structural organisation of their envelopes. Virus particles are no longer spherical but develop a convoluted profile and such virus particles are characterised in freeze-fracture replicas by the presence of ~30-nm wide and up to 0.5-μm long smooth linear ridges on E faces (Fig. 1c) and by a complementary arrangement of linear grooves on P faces (Fig. 1d). Instead of ~14-nm diameter intramembranous particles on E faces

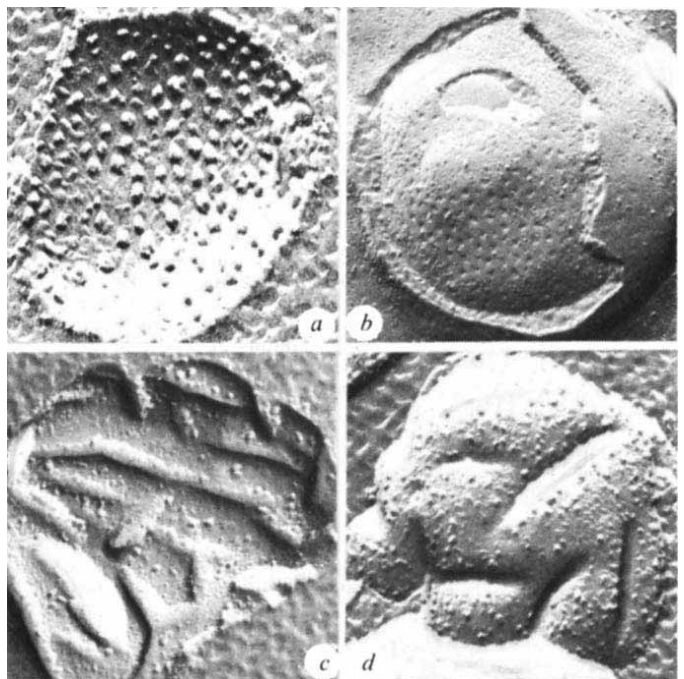


Fig. 1 Freeze-fracture replicas showing fractured Sendai virus particles at 4 °C (a, b) and at 37 °C (c, d). At 4 °C concave E fracture faces (a) display numerous ~14-nm diameter intramembranous particles and P faces a complementary arrangement of pits (b). At 37 °C concave E faces (c) of many virus particles now feature several ~30-nm wide smooth linear ridges and convex P faces (d) a complementary arrangement of linear grooves. ~9-nm diameter intramembranous particles are seen on the non-ridge regions of both P and E faces (c, d). *a* × 93,500; *b* × 51,000; *c* × 72,250; *d* × 82,875.

~9-nm diameter intramembranous particles can be seen on both P and E faces (Fig. 1c, d). These changes in the structural organisation of the viral envelope seem to be cell mediated and only take place when virus particles are bound to erythrocytes; free virus particles incubated and glutaraldehyde fixed at 37 °C remain spherical and display the same morphology as virus particles at 4 °C as in Fig. 1a and b).

Only virus particles having this altered morphology appear to be capable of fusing with the erythrocyte membrane; other virus particles which remain spherical never fuse and even after a prolonged incubation at 37 °C such spherical virus particles remain bound but unfused with the plasma membrane.

The smooth ridged regions of the viral envelope seem to be the initial site of fusion of the viral envelope with the erythrocyte membrane and the presence of these ridges allows specific sites of viral fusion to be recognised. Figure 2 shows a viral envelope which has fused and become completely incorporated into the erythrocyte membrane. In the conditions used there is no change in the distribution of erythrocyte intramembranous particles on either P or E fracture faces and at all times during agglutination and fusion intramembranous particles are randomly distributed.

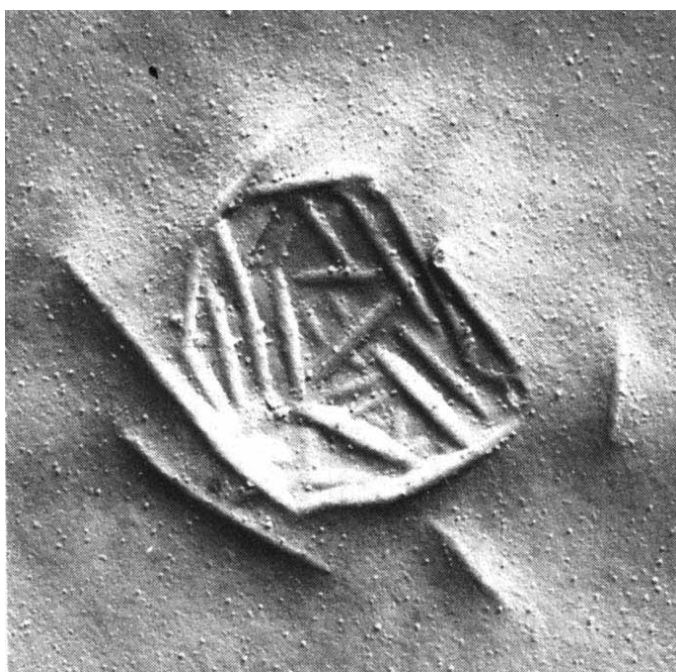


Fig. 2 Freeze-fracture replica showing the envelope of an unusually large virus particle which has fused and become completely incorporated into the E fracture face of an erythrocyte membrane ($\times 57,375$).

In summary, changes in the structure of the viral envelopes prior to fusion involve the loss of the ~ 14 -nm diameter intramembranous particles from E faces, the appearance of ~ 9 -nm diameter intramembranous particles on both P and E faces and a reorganisation of membrane components to produce the particle-denuded linear E face ridges. The biochemical changes which accompany these structural modifications are unknown.

Membrane fusion probably occurs when lipid bilayers in two opposing membranes are brought close enough to interact⁶. The particle-denuded linear ridge regions of the viral envelope probably represent such areas of lipid bilayer¹⁰. The observed membrane changes, therefore, are probably a specialised viral mechanism which allows fusion to take place as a result of interaction of the smooth ridge regions with the erythrocyte membrane. Although no gross changes in the distribution of erythrocyte intramembranous particles occurs, specific localised changes at the actual sites of fusion resulting in particle-denuded regions of erythrocyte membrane may occur possibly as a result of the viral neuraminidase.

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Cyclic AMP-dependent protein kinases from normal and SV40-transformed 3T3 cells

THE apparent molecular mechanism by which cyclic AMP regulates eukaryotic physiology is through the activation of cyclic AMP-dependent protein kinase enzymes¹. Two cytosolic protein kinase isozymes, called types I and II², have been described in mammalian tissues. They differ in their charge³, dissociability by salt³, capacity to undergo an autophosphorylation reaction³ and in their antigenic properties⁴. Evidence suggests that cyclic AMP regulates cell division. Intracellular levels of cyclic AMP are higher in normal than in transformed cells^{5,6}, and exogenous addition of cyclic AMP analogues to the culture medium inhibits the growth of transformed cells⁷. Cyclic AMP levels⁸, protein kinase specific activity⁹, and the relative amounts of types I and II isozymes¹⁰ vary during the cell cycle. A genetic lesion in lymphoma cells making them insensitive to cyclic AMP may be due to a defective protein kinase enzyme¹¹. We report here that cytosol from SV40-transformed 3T3 cells contains a type I protein kinase not found in BALB 3T3 cytosol. Chromatographic separation of these enzymes indicates that this isozymic difference is the result of an additional protein kinase regulatory subunit in SV3T3 cytosol.

Cell lines designated BALB 3T3 and SV3T3 originated from the BALB A₃₁ and SVT₂ lines, respectively (established by Dr George Todaro (NIH)); the Swiss 3T3 cell line was obtained from Dr Howard Green (MIT). The binding of cyclic AMP to protein was modified from the method of Gilman¹²; protein kinase assay was modified from Corbin and Reimann¹³; protein concentrations were estimated by the method of Lowry *et al.*¹⁴; and DEAE-cellulose chromatography of cytosol was modified from the method of Corbin *et al.*².

Initial studies indicated that the cyclic AMP-binding and protein kinase specific activities of cytosol fractions prepared from SV3T3 and 3T3 cells were similar, but not identical. The cyclic AMP binding activity (pmol ³H-cyclic AMP bound per mg protein) was 4.6 ± 1.1 for SV3T3 cytosol and 6.6 ± 2.7 for 3T3. The cyclic AMP dissociation constants (K_d), estimated from Scatchard¹⁵ analysis, were 2.3 ± 0.3 nM for SV3T3 and 3.3 ± 0.7 nM for 3T3. Cyclic AMP stimulated the SV3T3 protein kinase activity (pmol ³²P incorporated per mg protein per min) from 110 ± 30 to 710 ± 200 , and the 3T3 kinase activity from 170 ± 40 to $1,100 \pm 300$. These activity ratios (—cyclic AMP/+cyclic AMP) are approximately equal. Preincubation of crude cytosols at 60 °C before assay for cyclic AMP binding activity, however, indicated that the activity in 3T3 cytosol was more sensitive to heat denaturation than was the SV3T3 cytosol (Fig. 1). After 15 min at 60 °C, cyclic AMP-binding activity was

negligible in 3T3 cytosol, whereas SV3T3 cytosol still retained 25% of the activity. SV3T3 and 3T3 thus seemed to contain the same amount of cyclic AMP-dependent protein kinase activity, but these heat denaturation experiments suggested that the molecular structures of the protein kinase regulatory subunits were different. Chromatographic separation of these enzyme activities were carried out in order to examine this difference.

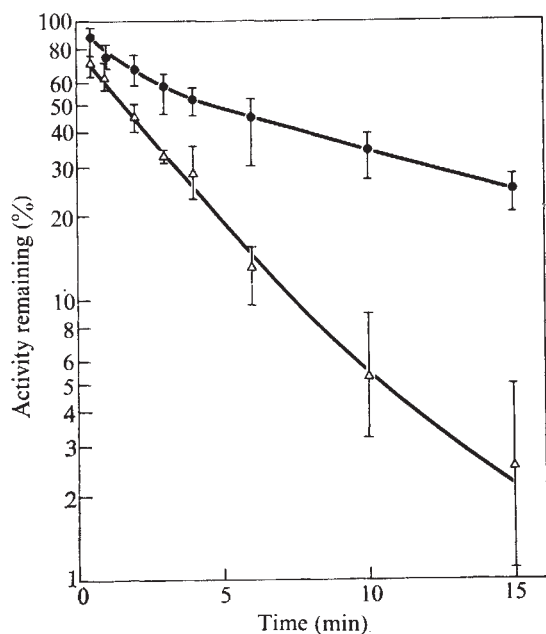


Fig. 1 The thermal stability at 60 °C of ^3H -cyclic AMP binding activity in SV3T3 (●) and BALB 3T3 (△) cytosols. Cells were grown in disposable glass roller bottles (Bellco) using Dulbecco's modified Eagle's medium supplemented with 10% calf serum. Approximately 2×10^6 cells were seeded into the bottles with 100 ml of medium, and 5% CO_2 was added; the bottles (three for SV3T3 and 12 for 3T3) were capped and placed in a roller bottle incubator (Forma) for 4 to 10 d (until confluency). The medium was then removed and the cells were washed twice with 20 ml of cold 0.32 M sucrose. The cells were scraped into 5 ml of sucrose and collected by centrifugation at 150g for 10 min. Cells were disrupted by sonication and subjected to the following consecutive centrifugations: 3,000g, 10 min; 27,000g, 15 min; and 100,000g, 40 min. The final supernatant (cytosol) was used in all experiments. Tightly capped tubes containing cytosols were incubated at 60 °C for varying amounts of time, cooled, and the binding activity was measured. The 200- μl binding assay reaction volume contained 50 mM sodium acetate (pH 4.0), 20 mM ^3H -cyclic AMP (16 Ci mmol $^{-1}$, Schwartz-Mann) and 60–120 μg of cytosol protein. After 5 h at 0 °C the binding component–cyclic AMP complex was separated from unbound cyclic AMP by Millipore filtration. Filters were counted as described by MacKenzie and Stellwagen¹⁶. Boiled blanks were used for assays of crude cytosol. The data for the SV3T3 cytosol are the average of six experiments, those for BALB 3T3 the average of three experiments. Range bars are shown.

Figure 2 shows the protein kinase and cyclic AMP-binding elution profiles obtained following DEAE-cellulose chromatography of BALB and Swiss 3T3 cytosols, and from two different SV3T3 clones. Both SV3T3 lines showed two peaks (Peaks 1 and 2) of cyclic AMP-dependent protein kinase activity which were coincident with peaks of cyclic AMP-binding activity. Peak 1 eluted at roughly 0.05 M NaCl and Peak 2 eluted at roughly 0.18 M NaCl. The activity ratio of peak 1 was increased by the addition of NaCl, while that of peak 2 was unchanged (data not shown). These properties suggest that peaks 1 and 2 are similar to the types I and II protein kinases found by Corbin *et al.*² in mammalian tissue cytosol. Both BALB and Swiss 3T3 lines lacked a peak 1 protein kinase but did contain a peak 2 protein kinase. The elution profiles of cytosols from logarithmically growing and from confluent

(quiescent) BALB 3T3 cells were indistinguishable (data not shown).

When SV3T3 cells were treated for 20 h with medium containing 10^{-3} M $N^6, O^{2'}$ -dibutyryl cyclic AMP (db cyclic AMP) and 0.5 mM aminophylline, the chromatographic pattern was considerably altered (Fig. 3a). Peak 1 contained no cyclic AMP-binding activity and had an activity ratio near unity, indicating that this protein kinase was independent of cyclic AMP. An additional peak containing 35% of the total cyclic AMP-binding activity and no protein kinase activity eluted after peak 1 and before peak 2. The amount of cyclic AMP-dependent protein kinase activity in peak 2 had decreased. When cytosol prepared from SV3T3 cells was treated with 10^{-4} M db cyclic AMP for 1 h (Fig. 3b), a similar elution profile to that found after the db cyclic AMP treatment of intact cells was obtained. When intact SV3T3 cells were washed just before collection with db cyclic AMP-containing medium, the elution profile was the same as that of cytosol from untreated cells. Thus, the db cyclic AMP treatment of intact SV3T3 cells was not a consequence of residual db cyclic AMP from the medium interacting with cytosols during sonication. The formation of a

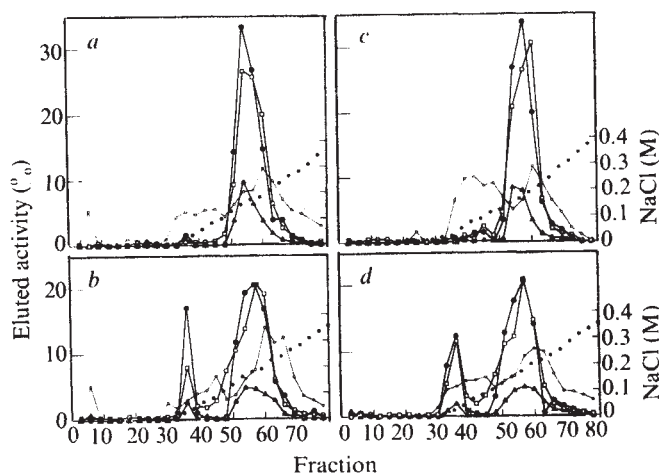


Fig. 2 DEAE-cellulose elution profiles of a, BALB 3T3; b, SV3T3; c, Swiss 3T3; and d, SV3T3_R cells. For a comparison of different samples, two identical columns were eluted from the same gradient mixer. DE-52 cellulose (Whatman) columns (0.9 × 10 cm) were equilibrated with 5 mM N -2-hydroxyethyl-piperazine- N' -2-ethanesulphonate (HEPES, pH 7.5) + 1 mM EDTA (buffer). After the application of 8–12 mg of cytosol protein, each column was washed with 30 ml of the buffer, and then eluted with a 60-ml linear salt gradient (0–0.4 M NaCl). The flow rates of each column were 10 ml h $^{-1}$; 75 fractions containing 1.2 ml were collected from each column. Conductivity measurements were used to determine the NaCl concentrations in the elution gradient. The 150- μl protein kinase reaction mixture contained 6.7 mM MgCl_2 , 20 mM 2-(N -morpholino)-ethane sulphonate (MES, pH 6.5), 300 μg histone (Type IIA, Sigma), 0.2 mM (γ - ^{32}P)ATP (about 20 c.p.m. pmol $^{-1}$), and 75 μl from a column fraction, with or without 10^{-6} M cyclic AMP. After incubating for 10 min (cyclic AMP present) or 20 min (cyclic AMP absent) at 30 °C, 100 μl of the reaction mixture was transferred onto filter paper squares (Whatman) and washed. γ - ^{32}P -ATP was prepared by the method of Post and Sen¹⁷. The Lowry *et al.* procedure¹¹ was modified to include HEPES buffer in the bovine serum albumin references when protein concentrations of column fractions were estimated. Activities shown are ^3H -cyclic AMP binding activity (□), protein kinase activity in the absence (△) and presence (●) of 10^{-6} M cyclic AMP, NaCl concentration (---), and protein (○).

cyclic AMP-independent protein kinase peak and a new peak of cyclic AMP-binding activity suggests that db cyclic AMP treatment of both intact cells and cytosol caused a dissociation of protein kinase holoenzyme into constituent catalytic and regulatory components. Treatment of cytosol from 3T3 cells with either cyclic AMP (Fig. 3c) or db cyclic AMP (Fig. 3d)

resulted in an elution profile different from that of untreated 3T3 cytosol and also distinct from the SV3T3 profiles. There was a decrease in the absolute amount of cyclic AMP-dependent protein kinase activity eluting at the peak 2 position, and the appearance of cyclic AMP-independent protein kinase eluting at the peak 1 position. The cyclic AMP-binding activity remained at the peak 2 position, and no peak of cyclic AMP binding appeared intermediate to peaks 1 and 2.

When the cyclic AMP-binding affinities and the cyclic AMP-dependent protein kinase specific activities of crude cytosol fractions from normal and transformed 3T3 cells were compared, no major differences were observed. This agrees with a previous study of protein kinase activities in normal and feline sarcoma virus-transformed cells¹⁸. Nevertheless, experiments testing the thermolability of the cyclic AMP-binding proteins in normal

BALB 3T3, while the patterns from two different SV3T3 clones were identical with each other, strongly suggests that the different kinase compositions resulted from virus transformation. These results are considerably different from those in which hepatoma cell lines were compared with normal rat liver^{16,20}. In these studies it was the tumour lines which lacked a type I kinase. A comparison of *in vivo* tissues with *in vitro* cells is not always valid, however.

db cyclic AMP treatment of intact CHO cells caused a dissociation of protein kinase holoenzymes²¹, as determined by Sephadex chromatography. DEAE-cellulose chromatography of SV3T3 cytosol following db cyclic AMP treatment of intact SV3T3 cells also indicated that protein kinase holoenzymes had dissociated into regulatory and catalytic components. Two cyclic AMP-binding peaks were observed when either intact SV3T3 cells or SV3T3 cytosol were treated with db cyclic AMP, while only a single cyclic AMP-binding peak was observed with the db cyclic AMP-treated 3T3 cytosol. Types I and II holoenzymes are apparently composed of identical catalytic components, but different regulatory subunits^{2,3}. The elution profile differences between 3T3 and SV3T3 cytosols may be explained by the presence of a single type of 3T3 protein kinase regulatory subunit, while the SV3T3 cells contained two different regulatory subunits. Some oncogenic viruses contain cyclic AMP-dependent protein kinases²². The additional cyclic AMP binding activity in the SV3T3 cell line may have been coded for by the viral genome. Alternatively, an infecting SV3T3 virus may have derepressed the expression of a type I protein kinase regulatory subunit in the host 3T3 cell.

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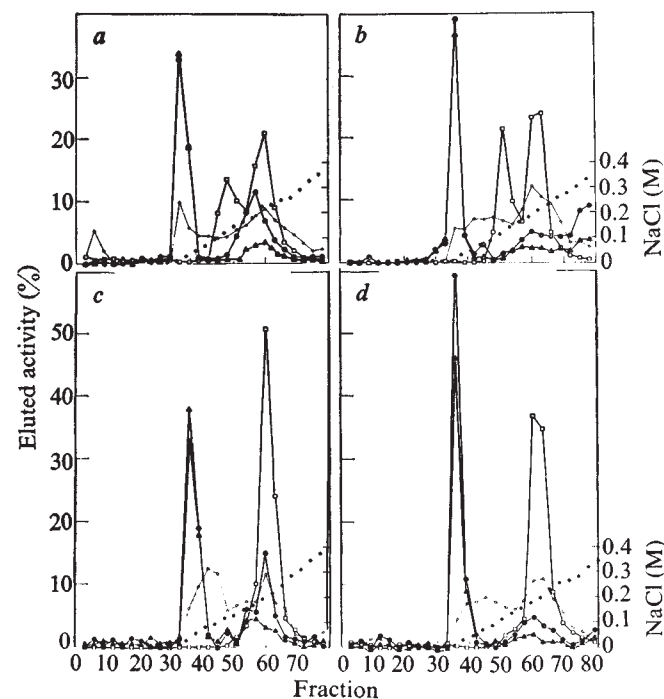


Fig. 3 DEAE-cellulose elution profiles of *a*, cytosol from SV3T3 cells treated with media containing 10^{-3} M db cyclic AMP + 0.5 mM aminophylline for 20 h; *b*, SV3T3 cytosol incubated with 10^{-4} M db cyclic AMP for 1 h; *c*, cytosol from BALB 3T3 cells incubated with 10^{-7} M cyclic AMP for 1 h; *d* and 3T3 cytosol incubated with 10^{-4} M db cyclic AMP for 1 h. Activities shown are 3 H-cyclic AMP-binding activity ($\square$), protein kinase activity in the absence ($\blacktriangle$) and presence ($\bullet$) of 10^{-6} M cyclic AMP, NaCl concentration ($\cdots$), and protein ($\circ$).

and transformed cells indicated that the cyclic AMP-binding activity from SV3T3 cells was substantially more heat resistant than the binding activity in 3T3 cytosol. Thus, in these studies, thermolability measurements seemed to be a more sensitive probe of differences between species of cyclic AMP-dependent protein kinases than either specific activity or K_d measurements. An increased heat stability of cyclic AMP binding activity *per se* is not a measure of tumorigenicity. The cyclic AMP binding activity of a highly malignant clone of neuroblastoma has been found to be more heat sensitive than that of a less tumorigenic clone¹⁹.

Elution profiles of the cyclic AMP-binding and protein kinase activities after DEAE-cellulose chromatography demonstrated major differences between cytosols from normal and transformed 3T3 cells. 3T3 cytosol contained a type II cyclic AMP-dependent protein kinase whereas SV3T3 cytosol contained both types I and II kinases (see Fig. 2). The fact that the Swiss 3T3 protein kinase elution profile was identical to that of the

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Partially supercoiled replication intermediates of R plasmid DNA are resistant to relaxation

A NUMBER of bacterial plasmids, including colicinogenic plasmids, F plasmids, and R plasmids have been isolated from *Escherichia coli* in the form of covalently closed circular (CCC) or supercoiled DNA-protein relaxation complexes which can be converted to a nicked circular (relaxed) DNA form by treatment with such agents as SDS, Pronase, or ethidium bromide^{1,2}. The relaxed molecules contain a single nick in a unique strand and at a unique site of the DNA duplex^{3,4}. These nicks occur at the origin of replication of Col E1 and R6K (refs 5, 6). We report here that the R plasmid ROR12 in *Proteus mirabilis* is replicated as a partially supercoiled DNA molecule, as has been observed for replicating molecules of SV40 and polyoma viral DNA⁷, mitochondrial DNA⁷, and the DNA of several plasmids^{5,6,8-10}. Replicating ROR12 molecules have a broad distribution of buoyant densities between the values of the CCC DNA and nicked circular DNA in an ethidium bromide-caesium chloride (EB-CsCl) density gradient. In addition, a substantial fraction

of replicating ROR12 DNA was found to have a density which was less than the value of the nicked circular form. These partially supercoiled replication intermediates of ROR12 DNA retain their supercoiled characteristics even when the DNA is isolated using lysis conditions which yield all of the non-replicating R plasmid DNA as relaxed molecules. This suggests that either replicating ROR12 molecules do not exist in the form of DNA-protein relaxation complexes as do the non-replicating plasmids or, if they do, the replicating molecules are not susceptible to relaxation in these conditions.

The R plasmid ROR12 is a round of replication mutant of the R plasmid NR1 (ref. 11). There are about four times as many copies of ROR12 per chromosome than there are of NR1. When isolated from *P. mirabilis*, the structure of non-replicating ROR12 DNA depends on the conditions in which the host cells are lysed¹². When the cells are lysed in SV buffer at pH 10.2 (0.15 M NaCl + 0.10 M EDTA, pH 10.2), all of the ROR12 DNA is in the CCC form and the DNA is not subject to relaxation by SDS, Pronase, ethidium bromide, or alkaline pH. But when the cells are lysed in SSP buffer at pH 11.0 (0.15 M NaCl + 0.015 M NaH₂PO₄, pH 11.0), all of the ROR12 DNA molecules contain a single nick in a unique strand of the DNA duplex and the majority of the linear strands seem to be attached to a proteinaceous cellular component¹². These findings suggest that

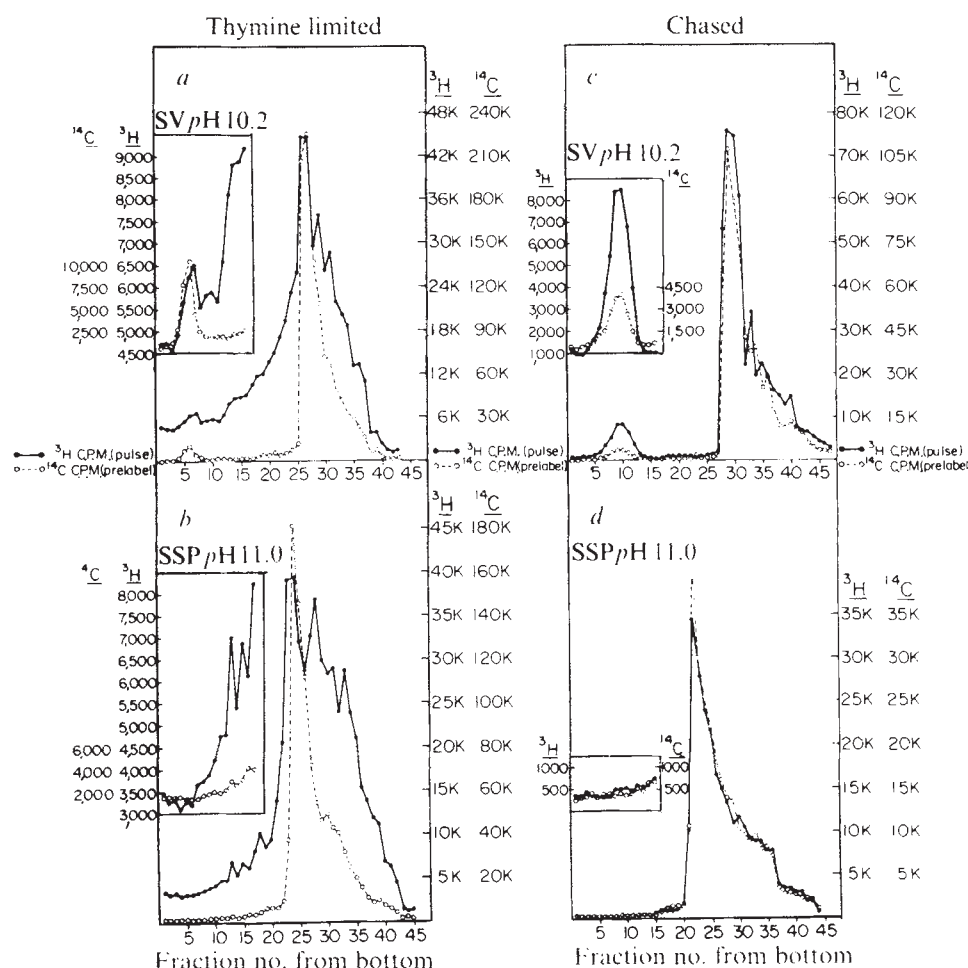


Fig. 1 Density profiles of uniformly labelled and pulse-labelled DNA in an EB-CsCl gradient. Pm15/ROR12 was cultured in M9 minimal medium (supplemented with 0.2% glucose, 10 μ g nicotinic acid ml⁻¹, 20 μ g tryptophan ml⁻¹, 0.5% vitamin free casamino acids, and ¹⁴C-thymine at 0.75 μ Ci per 2 μ g per ml) at 37 °C with shaking in a New Brunswick Gyrotory shaker bath. At an absorbance, A₆₅₀ of 0.5, 16 ml of culture was filtered on to a 100 mm S and S 0.45 μ m membrane filter, washed with 37 °C thymine-free medium, and resuspended in 100 ml fresh 37 °C medium containing ³H thymine at about 12.5 μ Ci per 0.03 μ g per ml (limiting thymine). After 15 min of incubation at 37 °C with shaking, 50 ml was collected (pulse sample) in ice-cold centrifuge tubes containing ice and sodium azide at a final concentration of 0.01 M. The remaining 50 ml was filtered, washed, and resuspended as above into 50 ml of thymine-free medium at 37 °C. After 5 min (to deplete thymine pools) unlabelled thymine was added to 100 μ g ml⁻¹, incubation was continued for 15 min, and the cells were collected as above (chased sample). Half of each sample (pulsed or chased) was washed twice by centrifugation and resuspension in SV pH 10.2 (0.15 M NaCl + 0.10 M Na₂EDTA, pH 10.2) and finally resuspended in 1.0 ml SV. The other half of each sample was washed and resuspended as above except in SSP pH 11.0 (0.15 M NaCl + 0.015 M NaH₂PO₄, pH 11.0). The samples were then lysed by addition of 10 μ l 25% SDS, 0.1 ml of Pronase (autodigested, 10 min at 80 °C, 20 mg ml⁻¹) and incubation at 37 °C for 2 h. This was followed by incubation at 65 °C for 30 min and

slowly drawing the lysate 20 times into a 1-ml pipette. 0.5 ml of EB (2.5 mg ml⁻¹ in TES; TES = 50mM each Tris, Na₂EDTA, NaCl, pH 8.0) and 2.09 ml TES were added and mixed gently, followed by 3.45 g of solid caesium chloride. The mixture was centrifuged at 23 °C in a Beckman 50 Tri rotor for 60 h at 35,000 r.p.m. The gradients were collected on to strips of Whatman 3 MM filter paper (1 inch wide with 1-inch squares marked on the strips) through holes punctured in the bottom of the tubes. The strips were washed three times in ice-cold 7.5% trichloroacetic acid and three times in 95% ethanol, and dried in an oven at 160 °C. The squares were cut off the strips into scintillation vials and counted with PPO-toluene (19 gm PPO per gallon toluene). ○, ¹⁴C-prelabel; ●, ³H-pulse label. a, Pulse-labelled cells, lysed in SV pH 10.2; b, pulse-labelled cells, lysed in SSP pH 11.0; c, chased cells, lysed in SV pH 10.2; d, chased cells, lysed in SSP pH 11.0. Inserts are tenfold magnifications of the CCC and intermediate density DNA regions.

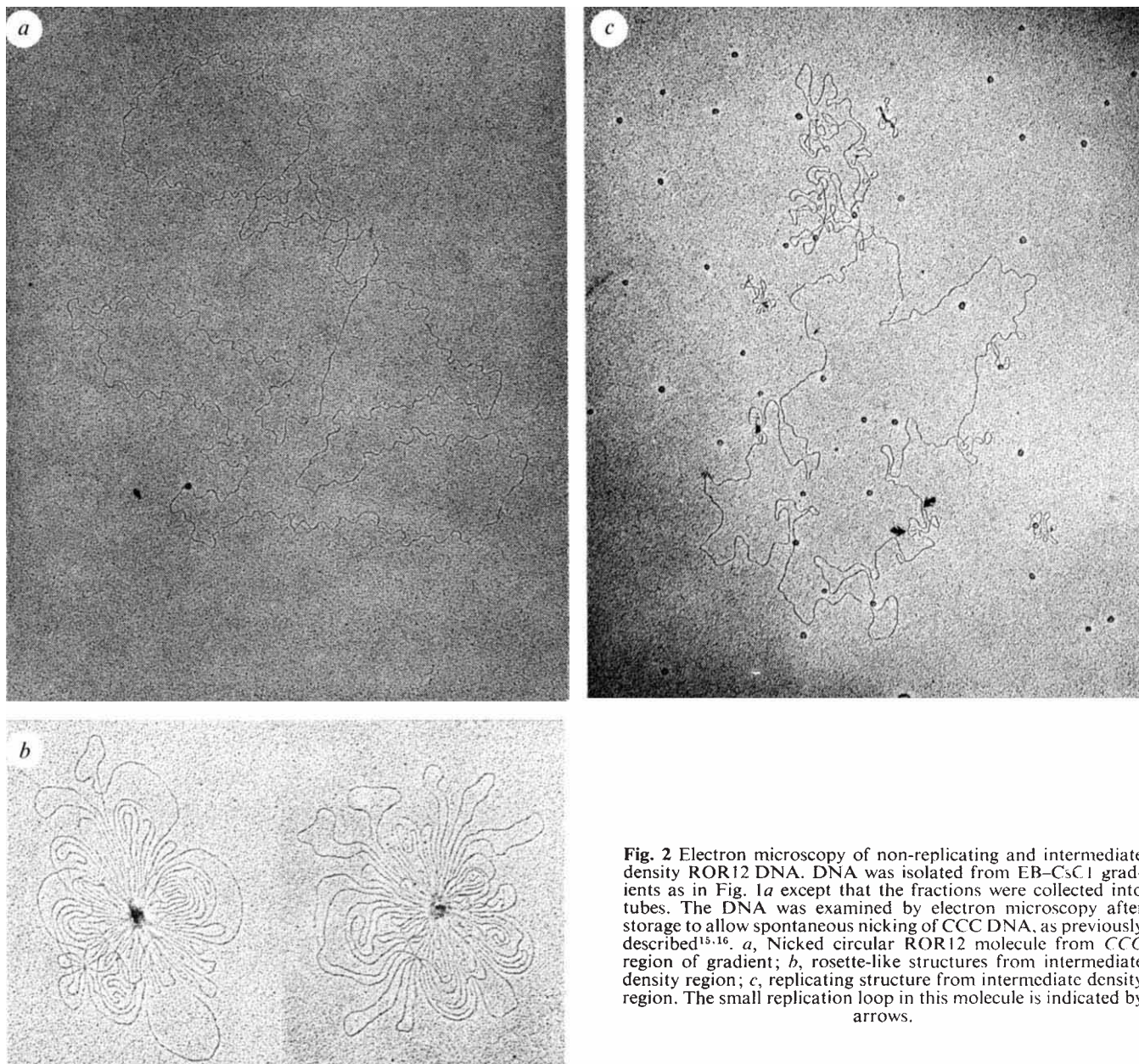


Fig. 2 Electron microscopy of non-replicating and intermediate density ROR12 DNA. DNA was isolated from EB-CsCl gradients as in Fig. 1a except that the fractions were collected into tubes. The DNA was examined by electron microscopy after storage to allow spontaneous nicking of CCC DNA, as previously described^{15,16}. *a*, Nicked circular ROR12 molecule from CCC region of gradient; *b*, rosette-like structures from intermediate density region; *c*, replicating structure from intermediate density region. The small replication loop in this molecule is indicated by arrows.

100% of non-replicating ROR12 DNA exists in the form of a DNA-protein relaxation complex in *P. mirabilis* cells. During lysis in SV buffer at pH 10.2, the protein seems to be dissociated from the DNA which remains in the CCC form. During lysis in SSP buffer at pH 11.0, all of the plasmid molecules are relaxed to the nicked circular form.

In *P. mirabilis* the fraction of plasmid molecules which is in the process of replication can be greatly increased by culturing a thymine auxotroph in medium containing limiting concentrations of thymine¹³. *P. mirabilis* strain Pm15/ROR12 was uniformly labelled with ¹⁴C-thymine and then pulse-labelled for 15 min in medium containing a limiting concentration of ³H-thymine (0.03 $\mu\text{g ml}^{-1}$). A sample of the culture was removed for DNA isolation and, after the addition of a high concentration of unlabelled thymine, the remaining culture was chased for 15 min before isolation of DNA. One-half of both the pulsed and chased samples was lysed in SV buffer at pH 10.2 and the other half of the samples was lysed in SSP buffer at pH 11.0. The lysates were centrifuged to equilibrium in EB-CsCl gradients in order to separate the DNA on the basis of the fraction in the supercoiled form and, hence, the extent of replication.

Figure 1a shows the density distribution of the DNA from

the sample which was lysed in SV buffer at pH 10.2. The ¹⁴C-prelabelled DNA is found in two bands which correspond to CCC plasmid DNA (fractions 4-8) and linear chromosomal DNA (fractions 25-37). A large fraction of the ³H-pulse-labelled DNA has a density between the values of the non-replicating CCC ROR12 DNA and the linear chromosomal DNA (referred to as intermediate density DNA), as has been observed for other replicating circular DNA molecules⁵⁻¹⁰. In addition, a substantial fraction of the ³H-pulse-labelled DNA has a density which is actually lower than the density of the linear chromosomal DNA (referred to as a light-shoulder DNA). Intermediate density DNA or light-shoulder DNA is not observed in ³H-pulse-labelled DNA from either an R⁻ culture of *P. mirabilis* which has been subjected to thymine limitation or from a culture of Pm15/ROR12 which was not subjected to thymine limitation. DNA hybridisation experiments and buoyant density analysis of purified light-shoulder DNA after removal of EB indicate that the light-shoulder DNA is ROR12 DNA (data not shown). In a reconstruction experiment using a mixture of nicked circular ³H-labelled ROR12 DNA and ¹⁴C-labelled *P. mirabilis* chromosomal DNA, the nicked plasmid DNA had a slightly higher density than the *P. mirabilis* chromo-

somal DNA in an EB-CsCl gradient, as would be expected on the basis of their densities which were determined in an analytical ultracentrifuge (1.712 and 1.700 g ml⁻¹, respectively)¹¹. Taken together, these observations suggest that the light-shoulder DNA is an intermediate in ROR12 DNA replication.

Figure 1b shows the density distribution of the DNA which was prepared from the thymine-limited culture which was lysed in SSP buffer at pH 11.0. In agreement with our previous observations¹², no ¹⁴C-prelabelled non-replicating CCC ROR12 DNA is observed when the cells are lysed under these conditions. The small ³H-labelled CCC band in fractions 4–8 in Fig. 1a is also not observed in Fig. 1b. The ³H-pulse-labelled DNA is, however, still present in the intermediate density and light-shoulder regions when the cells are lysed in SSP buffer at pH 11.0.

The ¹⁴C-labelled and the ³H-labelled ROR12 DNA isolated from the chased culture both band at the position of the non-replicating CCC plasmid DNA in the gradient when the cells are lysed in SV buffer at pH 10.2 (Fig. 1c). The increase in the fraction of plasmid DNA which was replicated during thymine limitation is reflected in the increase in the relative proportion of the ³H-labelled plasmid DNA band. When the chased culture was lysed in SSP buffer at pH 11.0, neither ¹⁴C-labelled nor ³H-labelled CCC ROR12 DNA was observed (Fig. 1d), in agreement with our previous experiments with non-replicating molecules¹². Since the plasmid molecules would have completed replication during the chase period, neither the intermediate density DNA nor the light-shoulder DNA is observed in Fig. 1c and d. The ³H-labelled DNA present in these regions in the DNA from the thymine-limited culture would have been 'chased' into non-replicating CCC molecules to produce the enlarged ³H-labelled ROR12 DNA band seen in Fig. 1c. These CCC molecules would have been relaxed when the DNA was isolated using SSP buffer, pH 11.0 (Fig. 1d).

When DNA from the ¹⁴C-labelled CCC plasmid band of Pm15/ROR12 cultures which have been subjected to thymine limitation is examined by electron microscopy after storage for several days to allow spontaneous nicking of supercoils, most of the DNA molecules are observed to be open circular structures which have contour lengths of ROR12 DNA (Fig. 2a). However, the majority (99/104 in one experiment) of the intermediate density DNA molecules are in the form of rosette-like structures (Fig. 2b). Some open circular structures containing small replication loops are also found in the intermediate density region (Fig. 2c) which have the contour length of ROR12 DNA. Owing to the twisting and overlapping of the molecules, the length of the DNA in the rosettes is difficult to measure accurately, but seems to be similar to that of replicating ROR12 DNA molecules. Rosette-like structures are also found in the DNA from the light-shoulder region. Whereas a minor fraction (8/31 in the experiment shown in Fig. 2) of the DNA from the ¹⁴C-labelled CCC plasmid band of Pm15/ROR12 cultures which have been subjected to thymine limitation appear as rosette-like structures, these structures are rarely observed in the corresponding CCC plasmid DNA band from either chased cultures or from cultures not subjected to thymine limitation. Similar results have been reproducibly obtained in several experiments which indicates that the rosette-like structures are not due to nonspecific aggregation of DNA and proteins under the conditions of these experiments (our work, in preparation).

These results indicate that ROR12 DNA replicates in the form of a partially supercoiled structure and that a substantial fraction of the replicating plasmid DNA actually has a density in an EB-CsCl gradient which is less than observed for the nicked circular form of the plasmid DNA. These replication intermediates remain in the partially supercoiled form during cell lysis conditions (SSP buffer, pH 11.0) which yield all of the non-replicating plasmid DNA as nicked (relaxed) structures. This suggests that whatever is responsible for producing the strand-specific nick in non-replicating molecules of ROR12 DNA under these lysis conditions does not affect the replicating plasmid molecules. Thus, either replicating ROR12 DNA does

not exist in the form of a relaxation complex, or, if it does, the complex is not capable of introducing the single nick into replicating plasmid molecules. Although the rosette-like structures which have been observed in the electron microscope have not been further characterised, it is interesting to note that these structures occur in the same density regions of the EB-CsCl gradient as the partially supercoiled replication intermediates.

The plasmids Colicin E1, R6K, and pSC101 have been isolated from *E. coli* as DNA-protein relaxation complexes^{1–3}. Relaxation of these molecules can be induced by treatment with SDS or EB. Replicating molecules of these plasmids have, however, been isolated from *E. coli* and have a density in an EB-CsCl gradient which is intermediate between the densities of the CCC and nicked circular forms of the DNA^{5,6,8,9}. Although not stated by the authors in these other investigations, these findings suggest that the replication intermediates of these plasmids are also resistant to relaxation by conditions which have been shown to induce relaxation of the non-replicating plasmid molecules. We have also studied the replication of the plasmid RSF2124 (Colicin E1 plasmid containing the ampicillin transposon)¹⁴ in *P. mirabilis* during thymine limitation, and have obtained results which are essentially identical to those described above for ROR12 DNA, including the presence of a light-shoulder replication intermediate.

In the cases of Colicin E1, which replicates unidirectionally, and R6K, which replicates bidirectionally, the site of the nick introduced by relaxation of the complexes has been found to be at or near the origin of replication of each of these plasmids^{5,6,8}. It has been suggested that relaxation complexes may function *in vivo* by acting as a swivel mechanism which allows unwinding of the parental strands during DNA replication¹. Alternatively, the relaxation complexes may provide the first nick which might be required for the initiation of replication. The results presented here (as well as data published by other laboratories) indicate that replicating plasmid molecules are not found in the form of relaxation complexes or that the complexes are not capable of introducing a nick into partially supercoiled replicating molecules. Thus, the function of relaxation complexes may be more probably involved with the initiation of replication rather than as a swivel mechanism during chain elongation, although other possibilities are not excluded.

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Mechanism of DNA synthesis inhibition by arabinosyl cytosine and arabinosyl adenine

1- β -D-ARABINOFURANOSYL CYTOSINE (ara-C) and 9- β -D-arabinofuranosyl adenine (ara-A) are potent antileukaemic and antiviral agents which inhibit DNA replication in various organisms¹⁻³. Two plausible hypotheses for their cytotoxic action centre on the replicative DNA polymerase: in model 1 the triphosphate derivative of ara-C or ara-A inhibits competitively the utilisation of the corresponding deoxyribonucleoside triphosphate in the DNA polymerase reaction, and in model 2 the analogue is incorporated into DNA and this lesion impedes synthesis by, for example, providing a poor primer for further chain elongation. Although both of these effects have been demonstrated *in vitro*, the target *in vivo* for the analogues has not been firmly established, in part because metabolites of these drugs inhibit a number of purified nucleic acid enzymes¹⁻⁴. Bacterial mutants with altered drug sensitivity could be decisive in determining the mechanism of inhibition and are the subject of this report.

Previous studies showed that ara-CTP inhibits replicative DNA synthesis in toluene-treated *Escherichia coli* and *Bacillus subtilis* and the purified DNA polymerases II and III but not I of these organisms⁵⁻⁶. We demonstrated that ara-CTP was also a substrate for the *B. subtilis* polymerases and concluded therefore that DNA chain growth was impeded by an ara-CMP residue at the 3'-hydroxyl terminus of the primer, as postulated by model 2 (ref. 4). Although the crucial enzyme for replication, DNA polymerase III (ref. 7), is less sensitive to ara-CTP than DNA polymerase II *in vitro*, even a low frequency of incorporation by polymerase III would result in the inhibition of propagation of the growing fork *in vivo*⁴. Substantiation of this mechanism by genetic means has been impeded by the inefficient growth inhibition of bacteria as compared to eukaryotic cells by ara-C and ara-A (refs 3 and 8).

We discovered that a DNA polymerase I mutant of *B. subtilis* had a very low efficiency of plating on ara-C or ara-A containing media, whereas the isogenic *polA*⁺ control was essentially unaffected by the highest concentration of drug tested (Fig. 1). (The genetic nomenclature is detailed in refs 4 and 9.) In other experiments, the analogues were shown to be bacteriocidal for *polA* mutants. The sensitisation to drug is caused by the DNA polymerase I mutation. Three independent *B. subtilis* *polA* mutants (*polA59*, *polA13* and *polA06* alleles) were similarly drug sensitive. DNA from a *polA06* strain was used to transform a strain containing the linked *mdh* marker⁴, and 11 of the 77 *mdh*⁺ transformants were *polA* and ara-C sensitive with the

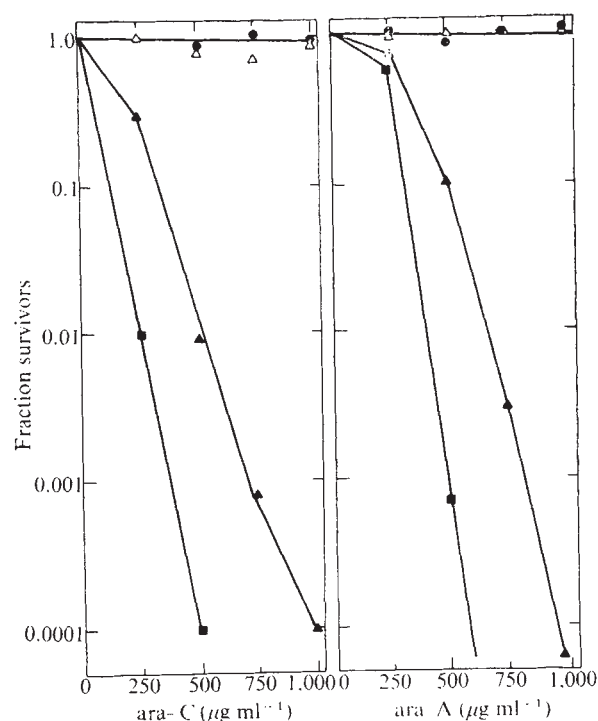


Fig. 1 Efficiency of plating on ara-C and ara-A media. *B. subtilis* strains BD311 (*polA*⁺), ●; BD314 (*polC26*), ▲; BD315 (*polA59*, *polC26*), ▲; and BD318 (*polA59*), ■; were spread on KML media¹⁰ containing the indicated drug concentration. After 1–2 d at 25 °C, the colonies were counted. The polymerase mutations were introduced by transduction with phage AR9 and strain BD311 is a *polA*⁺ sibling of strain BD318 (D. Dubnau, personal communication).

remainder being *polA*⁺ and ara-C resistant. Introduction of *polA* mutations into several *B. subtilis* strains always conferred drug sensitivity, although the extent of killing was strain dependent, particularly for ara-A.

A *polA1* strain of *E. coli*, H560 (ref. 11), was also sensitive to ara-C. In these experiments, filter disks impregnated with ara-C were placed on KML (ref. 10) agar plates spread with bacteria. Whereas 250 μg of ara-C caused a clearing zone in the lawn of strain H560, a *polA*⁺ isogenic control was not inhibited by even 2 mg of the drug, the highest amount tested. All 280 PI transductants of strain H560 to methyl methane-

Table 1 Efficiency of plating of *B. subtilis* mutants on ara-C and ara-A containing media

Strain	Relevant genotype	Efficiency of plating on:		Source of strain or ref.
		ara-C	ara-A	
HA101	wild type	1.0	1.0	13
F2	<i>polA59</i>	0.0003	—	13
F22	<i>polA59</i> , <i>polC22</i>	0.03	—	13
F25	<i>polA59</i> , <i>polC25</i>	0.00003	—	4
F26	<i>polA59</i> , <i>polC26</i>	0.09	—	4
F27	<i>polA59</i> , <i>polC27</i>	0.0001	—	4
BD311	wild type	1.0	1.0	D. Dubnau
BD318	<i>polA59</i>	0.0001	0.0007	D. Dubnau
BD191	<i>recB2</i>	0.009	0.1	14
BD191-1	wild type	0.9	0.7	This report
BD193	<i>recD3</i>	0.07	0.1	14
BD193-1	wild type	0.8	0.8	This report
BD194	<i>recA1</i>	1.0	0.7	14
BD224	<i>recE4</i>	1.0	1.0	14
BD246	<i>recG13</i>	1.0	1.0	14
GSY1028	<i>recB2</i>	0.006	0.1	15

The survival on 500 μg ml⁻¹ ara-C or ara-A was measured as in Fig. 1. Since the killing of F2 and its *polC* derivatives by ara-A was low and variable, these data were omitted.

sulphonate resistance (*polA*⁺) were ara-C resistant. The inclusion in the agar of 0.5 $\mu\text{g ml}^{-1}$ tetrahydouridine, an inhibitor of cytosine deaminase¹², increased the sensitivity of strain H560 fivefold and resulted in the inhibition of the *polA*⁺ control by 250 μg of ara-C.

The enhanced killing of *polA* mutants made it simple to test the prediction of both inhibition models that variation in drug resistance should be conferred by mutational alteration of DNA polymerase III. According to prediction, the plating efficiency on 500 $\mu\text{g ml}^{-1}$ drug for two *polA*, *polC* mutants of *B. subtilis* was about two orders of magnitude higher than for their *polA* parents (Fig. 1, Table 1); this difference was large enough to permit direct selection of *polC* mutants in reconstruction experiments. The other *polC* mutations tested had either no effect or caused increased killing by the drug (Table 1 and unpublished data). The alteration in drug sensitivity is not a result of an outside mutation since the *polC* mutants were selected as spontaneous hydroxyphenylhydrazinopyrimidine resistant mutants^{4,13}, and transfer of the *polC26* mutation to a *polA59* strain by transduction to form strain BD314 resulted in resistance to ara-C and ara-A (Fig. 1).

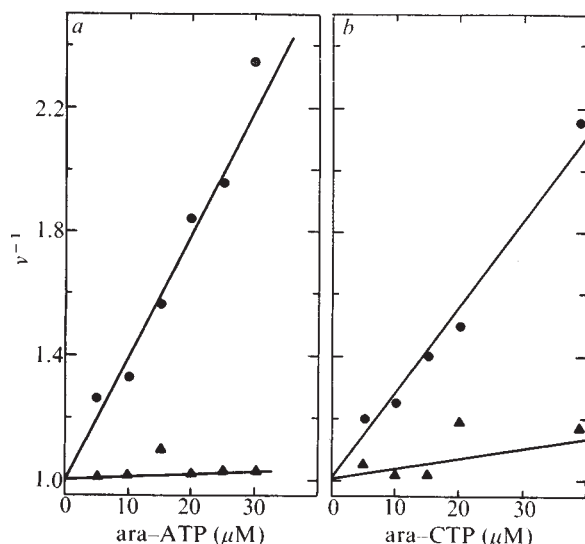


Fig. 2 Inhibition of wild-type and mutant DNA polymerase III by ara-ATP and ara-CTP. The reaction conditions were as described¹⁶ except the time of incubation at 30 °C was extended to 90 min; in *a*, the dATP concentration was 5 μM and ara-ATP was added to the indicated level, and in *b*, the dCTP concentration was 5 μM and ara-CTP was added to the indicated amounts. Each reaction contained 4 munits of wild-type, ●, or *polC22*, ▲, DNA polymerase III purified from *B. subtilis* strains BC26(F) and F22, respectively^{13,16}. Since synthesis truncated⁴ by omission of dATP was not inhibited by ara-ATP and that truncated by omission of dCTP was immune to ara-CTP, these values were subtracted in *a* and *b* respectively. The resultant reaction rate, *v*, in the absence of drug was set equal to 1.0. The calculated *K_i* values for the wild-type and mutant enzymes are 4 and 150 μM for ara-ATP and 2 and 17 μM for ara-CTP.

The *polC22* DNA polymerase was shown to be intrinsically drug resistant. The purified mutant DNA polymerase III was about an order of magnitude more resistant to both ara-CTP and ara-ATP than the wild-type enzyme (Fig. 2). *A priori*, the drug resistance could be the result of either an increase in the inhibition constant (*K_i*) for the drugs or a decrease in the *K_m* for the competitor deoxyribonucleoside triphosphate. Since we showed previously that the *K_m* for dGTP was the same for the *polC22* and wild-type polymerases¹³, and the *K_m* for dCTP was measured to be 0.3 μM for both the enzymes used here, the increased resistance of the mutant enzyme results from an increase in the *K_i*. The mutational alteration of the *polC22*

DNA polymerase near the active site¹⁷ must create a negative interaction with a portion of the arabinosyl compounds and hydroxyphenylhydrazinopyrimidines which is not shared by deoxyribonucleoside triphosphates. By analogy with the mechanism of resistance of the *polC22* polymerase to the arylhydrazinopyrimidines¹⁷, it is likely that this polymerase incorporates the ara-NTP residues inefficiently, rather than excising ara-NMP termini more rapidly or using these residues more effectively as primers.

The drug sensitivity conferred by the *polA* mutation could be a consequence of the role of polymerase I in the replication or repair of DNA⁷. Several other repair-deficient mutants of *B. subtilis* showed no sensitivity to ara-C and ara-A, but *recB2* and *recD3* mutants had a reduced plating efficiency on drug-containing medium (Table 1). The drug sensitivity is due to the *rec* mutations. Strain BD193 was constructed by introduction of the *recB2* mutation of GSY1028 and both strains were analogue sensitive (Table 1). Transduction mediated by phage AR9 of strains BD191 and BD193 to mitomycin C resistance or methyl methanesulphonate resistance rendered the resultant strains (BD191-1 and BD193-1) resistant to the drugs (Table 1). Although it is possible that these genes affect other cellular processes, the simplest conclusion is that the lesions in DNA caused by ara-C and ara-A can be repaired by pathways involving the *polA*, *recB*, and *recD* genes.

Several conclusions can be drawn from the data presented here. First, ara-A and ara-C probably act by way of the same mechanism, since mutations can cause a parallel increase or decrease in sensitivity to these drugs. Second, the marked effect of DNA polymerase mutations implies that metabolism of ara-A and ara-C to the triphosphate level is required for inhibition. Third, the correlated resistance *in vivo* and *in vitro* as a result of a mutational alteration of DNA polymerase III provides the best evidence so far that this enzyme is a primary target for the drug in the cell. Fourth, the simplest explanation of the enhanced sensitivity of *polA* and *rec* mutants is that the drug-induced lesion can be repaired at the DNA level, as required by model 2 but not by model 1.

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matters arising

Possible mechanism for biological action of lithium

FRAUSTO DA SILVA and Williams¹ have presented a theoretical chemical rationale for the postulate that lithium may have its pharmacological action by competition with magnesium²⁻⁴. But though they have dealt in detail with the role of ATP as a "masking" agent they have not considered the direct binding of lithium to the nucleotide.

Since lithium might interfere in the many biological processes which are magnesium dependent⁵ and since changes do occur in the tissue distribution of magnesium following lithium⁶ my colleagues and I investigated five magnesium-dependent enzymes and found that three were inhibited by lithium⁷. These enzymes seemed to be "Mg-ADP dependent".

We determined, by gel filtration on Sephadex G-10, the association constants of lithium and magnesium with either ADP or ATP both independently (K_i) and also in a solution containing both metals⁸. The constants determined in the (Li-Mg) solution were calculated *a*, assuming that the nucleotide complexed Li and Mg independently (K_{ii}) and *b*, assuming that the metals competed for sites (K_{iii}). All experiments were carried

Table 1 Association constants ($\log K_{ass}$) for complexes of Mg and Li with ADP:ATP in conditions described in the text^a

		ADP	ATP
$K(i)$	Mg	3.02 (a)	3.97
	Li	2.48 (b)	2.71
$K(ii)$	Mg	3.12 (c)	4.00
	Li	3.00 (d)	2.79
$K(iii)$	Mg	3.22 (e)	4.05
	Li	3.14 (f)	3.07

By *t* tests the following pairs are significantly different at $P < 0.001$: (a) against (e), (b) against (d), (b) against (f).

out at pH 7.4 in 0.108 mol l⁻¹ triethanolamine hydrochloride buffer. The results are shown in Table 1.

Our values for K_{ass} differ from those of Frausto da Silva and Williams but agree with other reports^{9,10}. But they are effectively "conditional constants" with respect to the nucleotide buffer-metals system at pH 7.4. We have concluded that there is preliminary evidence for a ternary complex of the Li-Mg-ADP type⁸ though this awaits confirmation by physical methods.

In relation to magnesium, ADP has a rather higher affinity for lithium than

ATP and if the lithium complex were a poor substrate this might have implications in the kinetics of ATPases during lithium therapy. In any case the increased binding of lithium induced by the presence of magnesium must indicate configurational or other changes which might affect the kinetics of any ADP requiring enzyme.

Our findings with biologically occurring nucleotides therefore suggest that the sequestering agent (ATP) may also be affected by lithium due to effects on the ATP-ADP equilibria. We might expect two loci of lithium action in nucleotide dependent enzymes; (O^- , N, O^- , O^-) coordinating sites on the protein and the nucleotide itself.

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WILLIAMS AND FRAUSTO DA SILVA REPLY—We accept the points made by Birch. The major point of our paper was to show how lithium could act on vesicles which contained transmitters even in situations where magnesium ions might be thought to be protective against any lithium effect.

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Uniqueness of plasminogen activators

THE report of Åstedt and Holmberg¹ relating their demonstration of the immunological identity between human urokinase and plasminogen activator from human ovarian carcinoma includes a quotation from the summary of one of our papers on plasminogen activators from transformed cells². Because of the interest in the role of plasminogen activators in tumorigene-

sis, we feel that it is important that there is no misinterpretation of our statement: "Neoplastic cells, whether transformed by oncogenic viruses or chemical agents, release a fibrinolytic factor not released by normal cells". In the text of our article, this sentence is qualified to indicate that by 'normal cells', we mean the parental primary cultures from which the transformants were derived. Our purpose in characterising the plasminogen activator from a transformed line was to determine whether or not it differed from the plasminogen activators known to be produced by normal mammalian tissues. We have, in fact, published data which demonstrate that the plasminogen activator which we purified from SV40 transformed hamster cells is immunologically identical to that produced by normal hamster lung cells and that it differs from the plasminogen activator(s) produced by hamster kidney cells³.

Although there is no doubt that there is a tantalising correlation between plasminogen activator production by cells *in vitro* and their ability to cause tumours in immune-competent hosts^{4,5} or in nude mice⁶, we feel that it is worth stressing once again that not all cultured cells which produce plasminogen activator are tumorigenic, nor do all cells capable of forming tumours in immune-competent hosts produce plasminogen activators *in vitro* (for example, Friend erythroleukaemia cells). In addition, as is confirmed by the work of Åstedt and Holmberg, the plasminogen activators produced by tumour tissues are not 'tumour-specific' proteins but probably reflect the expression of genetic information in 'inappropriate' cells.

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Temperature sensitive defect in human-hamster cell hybrid

IN a recent report Talavera, Basílico and Croce¹ reported incomplete complementation of a temperature-sensitive defect in 28S ribosomal RNA (rRNA) maturation after hybridisation of a Syrian hamster line, BHK 21/13 *ts422E* and LW SV40 transformed human cells. The incomplete complementation was expressed as a significant decrease in the growth rate of the hybrid cells as well as a reduced level of 28S rRNA production. In three of the hybrid populations tested, one contained exclusively BHK 28S rRNA and the other two showed a distribution of greater than 90% BHK 28S rRNA and only a very small proportion of human 28S rRNA. These results contrasted markedly with the situation with hybrids of the same BHK *ts422E* cells and two mouse lines in which good complementation was observed with the production of both mouse and hamster 28S rRNA at the non-permissive temperature (39 °C)². The distribution of mouse and hamster 28S rRNA ranged from predominantly mouse to predominantly hamster with some hybrids showing an equal distribution of both

those utilising pathway *b*, such as human cells. Furthermore, assuming that the BHK *ts* cells at 39 °C cannot provide all of the products necessary for maturation by pathway *b*, one could also account for the low yields of human 28S rRNA in the two hybrids analysed by Talavera *et al.*¹, which are presumed to contain actively transcribing human rRNA genes. Since the BHK *ts422E* mutant at the permissive temperature (33 °C) uses predominantly pathway *b*, characteristic of human HeLa cells^{3,4}, our interpretation leads to the testable prediction that at 33 °C *ts422E* should be able to provide the gene products necessary for human 28S rRNA production in the BHK × human cell hybrids F3 and F31.

The alternative explanations offered by us and by Talavera *et al.* are based on two different assumptions about the nature of the complementing function supplied by the L and HeLa cell partners in the hybrids with BHK cells. Given that the temperature-sensitive lesion in *ts422E* is pleiotropic in affecting both the cleavage at site 4 and the relative propensity for cleavage at sites 2 and 3, it is conceivable that the lesion is one which affects the conformation of the preribosomal particles in the

ing pathway in the hybrid cells.

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TALAVERA ET AL. REPLY—The appearance of 36S as well as 20S nucleolar RNAs in wild-type BHK (refs 1 and 2) indicates that the two alternative pathways of ribosomal RNA (rRNA) processing described by Winicov and Perry^{1,3} are probably an effect of differential rates of cleavage at the sites 2 and 3 (Fig. 1 of ref. 1). Pathway *B* could be absent in *ts422E* at 39 °C because of a partial effect on site 3 of the lesion that blocks completely the cleavage at the neighbouring site 4 (ref. 2). This would favour cleavage at site 2, resulting in a shift to pathway *A*. We therefore believe that pathway incompatibility⁴ alone cannot be taken as an explanation of the imperfect complementation found in hybrids between human cells and the BHK mutant *ts422E* (ref. 5).

Wt BHK cells as well as the mutant at 33 °C follow preferentially pathway *B* (characteristic of human cells). Thus, a perfect complementation of the *ts* defect in the human × *ts422E* hybrid should produce both at 33 °C and 39 °C the use of pathway *B*, which is the preferred pathway of both parent cells.

On the other hand the low amount of human 28S rRNA found at 39 °C in the hybrid populations F3 and F31 (ref. 5) can be explained according to Winicov and Perry⁴ only if one assumes that human rRNA processing functions are lost in the hybrids, as the parental human cells are not defective in rRNA processing. This seems unlikely; even if it cannot be completely excluded. As already discussed⁵, the selection and propagation of the hybrid lines should have strongly favoured cells which maintained the human chromosome(s) necessary to attain the highest possible level of 28S RNA.

For these reasons we believe that the defective cross-specific rRNA processing in our hybrids is more likely to be caused by specific binding differences in the formation of preribosomal particles, rather than by incompatibility between different processing pathways.

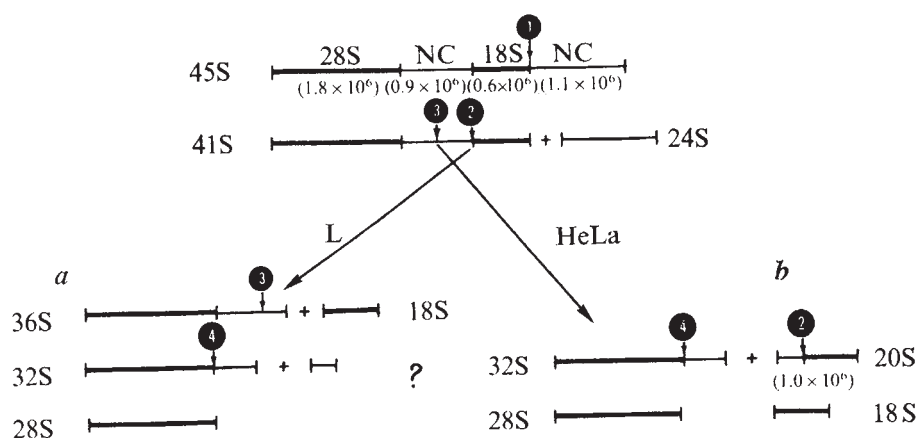


Fig. 1 The two pathways by which BHK *ts422E* can process rRNA.

28S rRNA types. We would like to offer a possible explanation for the poor complementation observed in the BHK human hybrids based on our own published data^{3,4}.

BHK *ts422E* can process rRNA by two alternative pathways as shown in Fig. 1: pathway *a*, which is characteristic of mouse L cells, and pathway *b*, which is characteristic of human HeLa cells. The BHK *ts422E* mutant at its non-permissive temperature (39 °C) uses pathway *a*, which in this case is defective at the 32S → 28S step. In hybrids of *ts422E* one might therefore expect complementation by gene products from cells exhibiting pathway *a*, such as mouse cells, but not from

regions of the relevant cleavage sites. The uncertainty is whether the complementing function restores the order of cleavages to pathway *b*, characteristic of the wild-type BHK cells, or whether the complementing function simply enables the *ts422E* cells to utilise pathway *a* more productively. The only essential feature of the complementation that was observed in the hybridisation experiments is that it restored the ability to make a proper cleavage at site 4, which is required for viability at the non-permissive temperature. Whether the other consequences of the pleiotropic lesion are also complemented cannot be ascertained without a more detailed analysis of the process-

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reviews

Black hole, meteorite or spacecraft?

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The Fire Came By: The Riddle of the Great Siberian Explosion. By J. Baxter and T. Atkins. Pp. 165+39 plates. (Macdonald and Jane's: London, October 1976.) £3.95.

ON June 30, 1908, there took place in a remote subarctic region of Siberia the event that for many years was regarded as the greatest meteoritic fall ever recorded. The general character of the occurrence was that of a stupendous explosion, almost certainly in the nature of an 'air burst'. For some decades now increasing doubt has been cast on the meteoritic hypothesis. As is well known, almost nothing has been too fantastic to have been proposed as an alternative. This book rehearses the story and the puzzle presented by it, and the authors advance their version of a particular class of solution.

News of the happening was slow in reaching the world at large and it made little impression when it originally did get abroad. The scientific community became aware of it only because an old newspaper report came to the attention of L. A. Kulik of the Mineralogical Museum in Petrograd (Leningrad) when he was preparing an expedition to locate meteorites that had fallen in USSR territory. After preliminary investigations in 1921, he and an assistant made an heroic exploration of the region in 1927, followed by several later expeditions. Much of what is known about the observable physical effects and of what was gleaned from surviving contemporary accounts and from the recollections of surviving witnesses we owe to this work. But there have also been Soviet ground and aerial surveys right into the 1960s. Many scientists in the USSR and elsewhere have worked on the interpretation of the evidence. All this is vividly described in the first half of the book, numerous photographs are reproduced and the appendices supply translations of reports of two expeditions.

The authors then discuss the problem that arose after it was generally appreciated that the evidence was incompatible with the fall of any known sort of meteorite. As they recall, as long ago as 1930 the English mathematician and meteorologist (not astronomer, as

Leonid Kulik, chief Soviet investigator.



stated), F. J. W. Whipple, and a few years later the Russian I. S. Astrapovich, suggested that the object concerned was a small comet. This remains the most generally favoured explanation, and the authors dismiss it much too casually. If the hypothesis is modified by supposing the object to have been a fragment of a comet that disintegrated at about the Earth's distance from the Sun, as comets are apt to do, and not just a mini-comet all on its own, then it does indeed appear as much the most plausible of the proffered explanations.

The authors allow themselves to be beguiled by the two speculations: that a spacecraft was concerned and that the explosion was atomic. That the explosion was such they contend from a comparison of its effects on the ground and in the atmosphere with those of atomic bombs exploded in 1945 and in tests before and since. For fairly generally accepted reasons, they discount the alternatives of an explosion caused by a body of antimatter or a black hole. As scarcely more than science fiction, the suggestion that an extraterrestrial spacecraft was involved has been around since about 1946. The authors take it seriously because there were reported sightings of a cylindrical missile-like object just before the explosion which, they think, implied two

abrupt changes of course, and because they think the conformation of the devastated area to indicate that the explosion came out of a "non-explosive shell". They go on to suggest that the explosion was a mishap to an atomic-powered spacecraft from some planetary system other than the solar system (although they seem ambiguous about this). The speculation seems to be irresponsible and to be suggested by the flimsiest of evidence. So it cannot be treated as a starting point for serious discussion of the plausibility of communication with extraterrestrial communities.

As is so often urged, we ought not to scoff at the general notion of some form of such communication, and we have to recognise how potential communicators could be physiologically, technologically, psychologically and in almost every other way different from ourselves. On the other hand, any speculation on the subject must proceed on the hypothesis that such beings would be restricted to matter the same as ours, obeying the same laws of physics. But then the concept of something approximately like an interplanetary space vehicle, using atomic power, being used for interstellar excursions is surely quite ridiculous.

This reviewer regrets having to make such remarks about a book on this amazing event that starts so well. Having accumulated so much fascinating material the authors could have sifted it into hard observations made on the scene by professional scientists, seismic and meteorological observations made elsewhere at the time and the reasons for associating these with the event, and the adequately corroborated elements in eye-witness accounts. They seem also to have the material for proceeding to some quantitative comparison of this evidence with the predictions of the various theoretical models that have been proposed. Had the authors done so—I cannot but think they would have reached very different conclusions—their book would have made even better reading than it does now, and it would have been a unique permanent contribution. □

W. H. McCrea is Emeritus Professor of Astronomy at the University of Sussex, UK.

Dynamic panorama of astronomy

Our Changing Universe: The New Astronomy. By John Gribbin. Pp. 160. (Macmillan: London, 1976.) £4.95.

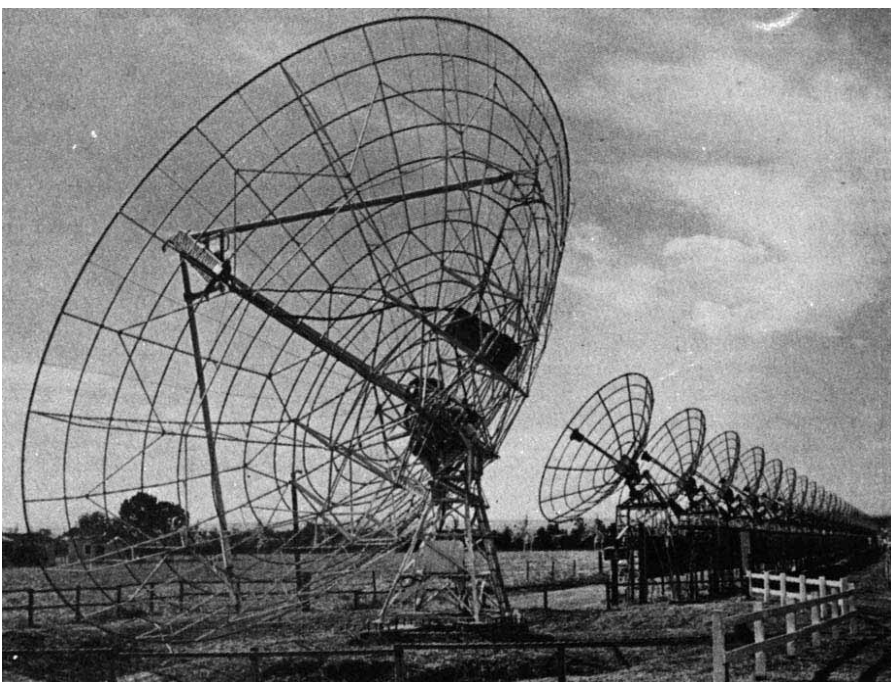
ASTRONOMY, in the doldrums thirty years ago, is now a thriving science, with a continuing march of discoveries that have transformed the static picture of past decades into an explosive and dynamic panorama. The immense changes in the science have been wrought by the development of the invisible astronomies that utilise observations in the non-visual ranges of the spectrum. Radio telescopes were the first to ruffle the calm waters of the optical astronomer, and these have been followed by X-ray satellites and a host of observation techniques based on the particle philosophy of modern physics. We now view a new universe, and one which Dr Gribbin sketches succinctly in his admirable book.

Beginning with radio astronomy, pulsars and quasars, we move on in a series of short chapters to cover the whole gamut of the modern scene—X-ray and neutrino astronomy, the significance of cosmic ray observations, the existence or otherwise of gravitational waves, the cosmic background radiation, and the question of whether the Universe is of the big bang, oscillating, or some altogether different type. In fourteen brief chapters covering some 66 pages of text, we have a brief but concise survey of the stellar and galactic universes.

There is also a chapter on the planets in the light of recent space probe observations, and another where Dr Gribbin gives his own views on what astronomy may be doing in the near future—a section where optical astronomy comes in for some comment. Finally there is an appendix, "Ice Ages, Man and Solar Neutrinos", in which there are some controversial ideas on a subject that is one of the author's consuming interests.

To concentrate most of the text on new observation techniques and their results was wise since they provide an ideal subject for a brief survey designed to be both relevant and exciting. The book is helped by a great number of well-chosen illustrations, some in colour, and all relevant to the text. There is an adequate index and a useful if restricted bibliography.

Of course, every reviewer is bound to have reservations on how an author apportions his space in a book of this kind—possibly more could have been said about BL Lacertae objects, on the electronic observing techniques now



used on optical telescopes, and at least a passing reference to that ingenious invention, the intensity interferometer—but there are bound to be personal preferences.

One wonders here and there about the level of previous knowledge expected of the reader: N-galaxies are mentioned but not specifically defined, spectroscopic principles are taken for granted, yet elsewhere the text could be understood even by a raw

beginner. Yet the general approach is such that no-one will get lost even though an equation appears as an integral part of one piece of descriptive writing. Every reader is bound to be swept along by Dr Gribbin's style, which is as dynamic as the universe he describes.

Colin A. Ronan

Colin Ronan is Editor of the Journal of the British Astronomical Association.

Basic guide to the galaxies

Exploring the Galaxies. S. Mitton. Pp. 206. (Faber and Faber: London, 1976.) £4.75.

THERE are three main readerships for astronomy books: the professional astronomers, the amateur with a serious interest who makes his living elsewhere, and the more casual reader who has a passing (if sometimes quite deep) interest and wants to know the story of what is going on without being swamped by technicalities. Since it is rare that a book can cater satisfactorily for any two of these markets, let alone all three at once, it is well for an author to have his intended readership clearly in mind, and Mitton's *Exploring the Galaxies* is described on the cover as intended for the non-specialist, a "guided tour . . . which brings the reader to the threshold of contemporary research". So this is definitely not a book for the professional astronomer, who has already progressed beyond those fringes, and given its sober design

and the inclusion of diagrams of the kind familiar to readers of *Nature* (indeed, some culled from these pages) seemed therefore aimed at the 'serious' amateur, rather than the casual reader best hooked by pretty pictures. Unfortunately, the author has failed to distinguish clearly between these two markets, with the result that his text steers a sometimes uneasy course between superficiality and technicality, dropping anchor in neither port.

The book certainly starts simply, with a potted account of the history of astronomy—but it's not another account of this history that a prospective reader looks for in any new book on galaxies. Finding his feet with a chapter on instrumentation (but sadly dismissing X-ray telescopes in one paragraph) Mitton gets to grips with the basics of galaxies, their structure and the distances to them at a level which assumes some knowledge of physics, if only at the school rather than university level. At his best in describing recent work on giant radio sources, 'tadpole' radio galaxies and radio studies of QSOs Mitton falls down somewhat on the grounds of readability, producing such tortuous con-

structions as "it can be seen that at the present time it is not known with any certainty . . ." where a simple "we don't know . . ." would do. I was also disappointed to find very little stress placed on the increasing evidence of links between QSOs and active galaxies.

Overall, the book would be of interest to its intended market, but as such it must face comparison with such accepted standard works as Shapley's *Galaxies* (revised by Paul Hodge only a few years ago), Baade's *Evolution of*

Stars and Galaxies and *The Milky Way*, by Bok and Bok. As a basic guide to galaxies for someone with an interest in astronomy, Mitton's book has no major flaws; unfortunately, however, when looked at against the opposition it is top of the second division rather than a big league competitor.

John Gribbin

John Gribbin is a Visiting Fellow at the Science Policy Research Unit, University of Sussex, UK.

Sense of time

An Inventive Universe. By K. G. Denbigh. Pp. 220. (Hutchinson: London, 1975.) £3.75.

DR DENBIGH, Principal of Queen Elizabeth College, University of London, is a scientist who has managed to fulfil the duties of his post without abandoning his own academic activities. A distinguished thermodynamicist he has long had a profound interest in the subject of time, both in its philosophical and its scientific aspects. He has read widely and thought deeply about it and other fundamental concepts in science. Since he also has the gift of writing clearly and concisely, his latest book, which is addressed to a wider audience than most of his previous publications, deserves a warm welcome and it is to be hoped that many will read it.

Denbigh believes, in the widest sense of the term, in the evolutionary nature of the universe. Although he never refers to A. N. Whitehead specifically, he can be regarded in this respect, at least, as one of his followers. Whitehead maintained that our whole conception of knowledge has been vitiated by the assumption, going back to the Greeks, that transition and creation are 'inferior' to changelessness and invariance.

In Denbigh's short book there are five chapters of about 175 pages in all, supplemented by some 35 pages of notes and references. The first, and longest, chapter is on our construction of the concept of time, which he points out depends specifically on mental processes, despite its intersubjective character. Much of physics, however, is based on what he calls "a sort of pared down time concept", involving only the notion of a linear sequence and a quantitative scale. Consequently there is considerable divergence of opinion concerning the anthropomorphic nature, or otherwise, of time's arrow and time's transience. Although Denbigh recognises the inadequacy of the B-theory standpoint (to use McTaggart's well-known terminology), which denies objectivity to the distinctions we make

between past, present and future, he takes the A-theorist Hans Reichenbach to task for using Heisenberg's uncertainty principle as the basis of an objective 'now'. Denbigh's own leanings are towards the A-theorists and he repudiates the claim of those B-theorists who cite relativity in their support, since there is nothing in relativity that necessitates the B-theory standpoint. He concludes that the sense of time which we construct out of our conscious awareness is not confined to ourselves but provides an important link between the mental and the physical.

Chapters 2 and 3 are devoted, respectively, to dissipative processes and 'formative' processes. The former are associated with the law of increasing entropy, and the latter with the evolution of organisms and tissues of increasing complexity. These two distinct trends in nature are not incompatible for, whereas entropy is concerned with orderliness (and its decay), biological evolution is concerned with organisation. To elucidate the difference between these ideas Denbigh introduces the concept of "integrability", which he suggests may be analysed mathematically with the aid of graph theory. It is to be hoped that he will eventually find time to develop this stimulating train of thought more fully.

In Chapters 4 and 5, Denbigh discusses determinism and whether genuinely new things can come into existence during the course of time. He is a firm believer in the continual and natural evolution of novelty. At the end of the book he returns to the concept of time, stressing both its relation to consciousness and its intersubjective character. He argues that the totality of consciousness in the universe tends to increase, and he believes that a kind of principle of 'complementarity' (although he does not call it that) between the concepts of physics and chemistry and those pertaining to ourselves as 'persons' (rather than 'things') must be invoked if we are to understand the role of man in the world-order.

G. J. Whitrow

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Immunological cookery

Practical Immunology. By L. Hudson and F. C. Hay. Pp. xvi+298. (Blackwell Scientific: Oxford, London, Edinburgh and Melbourne, 1976.) £6.50.

THIS book is required in the laboratories of all those who practise the mysteries of contemporary experimental immunology. It is clearly written, adequately illustrated and very useful.

The authors are research workers who have, in addition, considerable experience of teaching both practical and theoretical immunology. It seems from their book that they enjoyed what they were doing, that they did it well and wished to communicate their enthusiasm and competence to others.

In the contents list to the book there are a series of rather arbitrary headings such as "Initial preparations", "Cellular dynamics *in vivo*", "Antibody interaction with antigen", and "Advanced techniques in cellular immunology". But in fact the authors have compiled a list of the main ingredients of immunological cookery and then gone on to provide a series of recipes for their use. The emphasis is avowedly on the cellular components of immune responses as the authors believe that this represents a good starting point from which consideration of the humoral aspects of immunity can develop.

The title of the book is misleading in that it seems to embrace the whole practice of immunology, whereas it is restricted almost entirely to description of the practicalities of avant-garde experimentation. Thus there is hardly any consideration given to parasite immunology except, surprisingly, the Wasserman test; nor to histopathological evaluation of results.

This book, held in conjunction with that on *The Immune System: a Course on the Molecular and Cellular Basis of Immunity* by Hobart and McConnell (Blackwell, 1976) and the older title *Essential Immunology* by Roitt (1974) should equip any budding immunologist with a knowledge of what has been done, how it was accomplished and what it all means. All that would be required to complete this paper armamentarium would be a text on "What to do next in Immunology". That would be useful.

A. J. S. Davies

A. J. S. Davies is Professor of Immunobiology in the University of London, working at the Chester Beatty Research Institute, London, UK.

Trends in Soviet science and design

Soviet Science, Technology, Design: Interaction and Convergence. By Raymond Hutchings. Pp. xii+320+8 plates. (Oxford University: London and New York, May 1976. Published for the Royal Institute of International Affairs.) £12.

THE idea of analysing the evolution of the interactions of science, technology and design in the Soviet Union is interesting and worthy of research, but the present study by Dr Ramond Hutchings must be regarded as a rather idiosyncratic first attempt. His book contains some factual material, notably on design, which will be new to most readers, and a number of suggestive insights into aspects of Soviet technological and design policy. But substantial sections contribute little to the existing literature in English on Soviet science and technology and, more seriously, the study is weakened by inadequacies of a conceptual and theoretical order.

Dr Hutchings discusses science, technology and design in isolation and then concludes with a brief consideration of their interrelationships. Chapters are devoted to the organisation and planning of science and the trends in expenditure on research and development in the post-war years, covering aspects of Soviet science treated in greater detail elsewhere, notably in the OECD study *Science Policy in the USSR* (1969). The chapter on finance is particularly sketchy and takes no account of the valuable studies of Louvan Nolt-ing of the US Department of Commerce. Consideration of some strands of technological policy is followed by a brief account of the problems of technical innovation. In a chapter on 'design as an institution' it is argued that following early interest symbolised by the activity in the 1920s of VKhUTEMAS (the Higher Technical Artistic Studios), a Soviet equivalent of the Bauhaus, interest in design suffered neglect until the early 1960s when a new specialised institute, VNIITE (All-Union Scientific Research Institute of Technical Aesthetics), was established. In describing this interesting organisation the author draws on his direct personal experience.

Finally, chapters are devoted to characteristic features of design in the USSR and the forces which have conditioned them—for example, the well known bias towards grandiose structures, and the influence of ideological symbolism. It is in this section that the author's rather idiosyncratic and eclec-

tic approach is most apparent, although at times his speculations on Soviet design peculiarities are stimulating and amusing. There are some minor factual inaccuracies, in part stemming from the fact that developments in the past five years are treated less thoroughly than for the 1960s.

The main thesis of the book is that science, technology and design in the Soviet Union are beginning to interact with increasing force in spite of pressures, primarily political and ideological, tending to maintain their relative separation, and that the forms of interaction are showing a tendency to converge with those typical in the West. Moreover, design, it is argued, has until recently, been the weakest link in the chain. Although much evidence in support of these propositions is adduced, there is a notable absence of serious theoretical treatment of the general processes of interaction of science, technology and design under capitalism and socialism and at different stages of industrial development. The concepts 'technology' and 'design'

are themselves defined with insufficient rigour; the former is used loosely to embrace technical sciences, engineering and technical artefacts, whereas 'design' in the main corresponds to activity in the sphere of aesthetics, rather than engineering design (although the author at times slides from one to the other). Thus, consideration of design focuses on VNIITE and little attention is devoted to the extensive network of design and project offices which forms a vital component of the Soviet research and development system. More rigorous treatment of the theoretical framework of this study would have made it a more weighty contribution to our understanding not only of Soviet society, but also of the exciting implications arising from the general tendencies of development of science, technology and design in the second half of the twentieth century.

Julian M. Cooper

Julian Cooper is a Research Fellow at the Centre for Russian and East European Studies, University of Birmingham, UK.

Notions of mechanism in biology

The Problem of Life: An Essay in the Origins of Biological Thought. By C. U. M. Smith. Pp. xxiv+343. (Macmillan: London and Basingstoke, May 1976). £10.

THIS is a gallop through biological history with an eye open for certain views of the passing countryside. It might have been called *The Triumph of Reductionism*, and our author plots the course of notions of mechanism in biology from ancient times to the present. The views of the countryside that attract him are those that show how man's idea of nature is determined by the society in which he lives. 'Biology' had a greater meaning when self-moving things were thought to be alive, or when the Greek natural philosopher, living in a *polis*, used 'political' explanations of the behaviour of animals.

The growth of technology provided another analogy with which to explain biological phenomena, and we are presented here with a biologist's view of the evolution of technology, mutations being selected for and against; such evolution produces a certain kind of society, and the society produces ideas of a certain sort, including those of the natural world. It is certainly

true that the history of science is largely the history of the knack of asking answerable questions of nature. This does not invalidate the unanswered questions, but the answered questions certainly help to mould the mind of the enquirer; this generates a bias in the new questions and the new people who ask them. To a certain extent science and perception of nature are thus 'culture bound', but very often the historian finds that scientific ideas are transmitted through societies, not generated by them, as the biologist or politician may be tempted to think.

The author's awareness of the limits of perception, of the urge of the mind to impose patterns on the perceived, and of the orientation of perception by culture leads him into early chapters on imagination, anthropology and language that are somewhat thin and not entirely related to the rest of the book. The level of scholarship in a book of such broad scope inevitably falters in places. In particular anatomy and Vesalius are inadequately dealt with, and there is some misinterpretation of the position of Borelli and the later animists. Nevertheless, the author's view of history is held with a firmness that provides a coherence and freshness that are particularly valuable in a survey of such a long historical period.

R. French

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Key discovery

Chemical Transmission of Nerve Impulses: A Historical Sketch. By Z. M. Bacq. Pp. 106. (Pergamon: Oxford and New York, 1975.) £6 net.

THIS translation of Professor Bacq's book was distributed by the publishers free of charge (in a paper-bound version) to 500 participants at the Dale Centennial Symposium of the Physiological Society in 1975. It is a pity that the hardback volume now published is so expensive and that the paperback version has not been made generally available, because the book is a delight to read and is more important than its small size implies. The discovery of the chemical transmission of nervous impulses must surely rank as one of the major discoveries in the biological sciences and so a brief account of the experiments and personalities behind the discovery is very welcome. Professor Bacq was himself involved in some of the early work with Cannon, Fredericq and Dale, and so his book is of especial value. He frankly admits that he is writing a personal view and I suspect that others who were involved in the research would not always agree with him. The virtue of this slightly subjective view of history, however, is that it involves the reader in the spirit of the time and gives him a real feeling for the excitement of discovery.

The first two very short chapters introduce the topic for the non-biologist; then we are told a little about the early workers who clearly expressed the hypothesis of chemical transmission but were unable to do experiments that satisfied the sceptics of the time.

In his description of the discovery of cholinergic transmission, the author raises a few doubts about some aspects of the autobiographical sketch which Loewi wrote in 1960 (at the age of 80). Bacq goes on to state: "The great merit of this exceptional man [Loewi] was his ability to follow a logical course and to accumulate convincing experimental evidence without paying too much attention to the criticisms of his theory". A few of Feldberg's beautiful experiments are then described, which are as convincing as any of Loewi's and which were carried out on the living animal.

Credit for the first experiment which showed that a chemical substance is involved in transmission of impulses from one nerve to another nerve (in the superior cervical ganglion) is given to Kibjakow (1933). Kibjakow's elegant experiment did not, however, throw any light on the nature of the substance released from the preganglionic fibre; it was Feldberg and Gaddum

who proved that it is acetylcholine. Bacq's account of transmission to striated muscle is strangely incomplete, for he does not mention the critical experiments of Dale, Feldberg and Vogt (1936) in which they identified acetylcholine in the perfusates from several voluntary muscles after stimulation of the motor nerves.

The account of the author's own experiments with Cannon on adrenergic transmission is not only fascinating but serves to correct those who judge Cannon's work only by his entanglement in the erroneous hypothesis (attributed by Bacq to Rosenbluth) concerning 'sympathin E and I'.

Perhaps the most amazing story in the book is to be found in the chapter describing the opposition by Eccles and Heymans to the idea of chemical transmission. The reader becomes a little sceptical about Eccles' use of Popper's philosophy to 'explain' his reluctance to accept sound experimental evidence over almost a decade.

Bacq's book should be read by all students of physiology, especially those intending to do research in the neurosciences. It is not, and does not pretend to be, a complete and objective history of the subject. One can only hope that this vivid account will provoke others who participated in the research to write down their recollections. Then, one day, the full story of this key discovery of modern biology will perhaps be written.

A. D. Smith

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Classic physics

Three Phases of Matter. By Alan J. Walton. Pp. xiii+492. (McGraw-Hill: London and New York, June 1976.) Hardcovers £15; softcovers £5.95.

THIS text is suitable for students embarking on science courses with a major or minor element of physics at University (or equivalent) level. It had its origins at Sussex where—I must here declare my interest—I worked briefly with the author in a lecturing double-act. Many of the arguments developed in the book had already taken shape then (1969) but the experience of the Open University, with its disciplines of clarity of presentation, has had a strong and beneficial effect.

The subject matter is the classic, if recently less fashionable, one of solids, liquids and gases. The style (quasi-conversational he calls it) is inimitable Walton. It is used to convey the impression that the author stands outside the 'establishment' as a sort of wise uncle always trying to bring an argu-

ment down to earth by relating it to everyday experience. "As is often the case," he says, "one can learn much from analogues." In fact the dependence on analogues and other physical models is very great.

Much basic groundwork is covered in the first three chapters (the p - V - T surface, characterising atoms, temperature) before the three phases (three chapters on gases, four on solids, two on liquids) are discussed. Very little previous physical knowledge is assumed or needed and the mathematics is kept to a minimum, although even that minimum is in places demanding as, for example, in the one-dimensional coupled oscillator model of a solid. There are liberal supplies of exercises and problems, with comments and/or answers.

The extreme care with which physical ideas are usually introduced and used is a feature of the presentation. I can't say that I would always agree with the author, and I feel that most readers will find parts which they find unnecessarily idiosyncratic and perhaps condescending. I read the book from cover to cover and I liked it. I hope there is a market for it.

D. S. Betts

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Nucleic acids revised

The Biochemistry of the Nucleic Acids. Eighth edition. By J. N. Davidson. Pp. xii + 420. (Chapman and Hall: London, October 1976.) Hardcover £8.50; paperback £4.50.

THE late Professor Davidson wrote seven invaluable editions of this book over twenty years of exciting discovery in nucleic acid biochemistry. The eighth edition by four of his former students and colleagues at Glasgow (R. L. P. Adams, R. H. Burdon, A. M. Campbell and R. M. S. Smellie) packs a mine of information into a volume that can still be put into a coat pocket. It emphasises the enzymology of nucleic acids with a sound foundation of their structural analysis and physico-chemical properties. Some chapters—especially that on the replication of DNA—form really advanced reviews. An excellent feature is the skill and authority with which important properties of prokaryotic and eukaryotic polymerases are compared. The book is copiously documented with many important references, has few errors or ambiguities in proportion to the wealth of its content, and is priced very reasonably, especially in the paperback edition.

Although this new edition will follow its predecessors in being an important source book in nucleic acid biochemistry, the role of the book may perhaps be changing. Nucleic acids were, for many years, treated poorly in even the best general biochemistry texts. Today, such texts may contain excellent sections on nucleic acids and Davidson's book is no longer needed to introduce students to the field. It still has no equal in providing the necessary background for those who are contemplating or beginning a research project in nucleic acid biochemistry.

Yet it is a pity that the eighth edition has lost the freshness of the first and does not bubble with the excitement and imagination that is still typical of much nucleic acid research. Some sections—for example, those on DNA sequencing or protein synthesis, are quite disjointed, presumably as a result of too much scissors and paste. There is no glimmer of the rich prospects opened up by the ability to recombine DNA *in vitro* and to clone the artificial recombinants. Several good electron micrographs of nucleic acids are shown, but the techniques are not outlined, and the value of electron microscopy is not emphasised enough. The relationships between nucleus and cytoplasm as may, for example, be illustrated in histone biosynthesis are

not explained. The relevance of 6-O-alkylation of guanine to chemical mutagenesis is not mentioned. Indeed, mutagenic screening tests to identify potential environmental carcinogens are not discussed.

In view of its reasonable price, one can forgive most of the cramped black-and-white diagrams. But some are so bad they cannot be excused. In particular, the numbered purine and pyrimidine ring structures are so tiny that they can hardly be read. Certain diagrams of replicating *Escherichia coli* DNA do not show two-directional replication and one shows linear chromosomes. A bacterial cell is drawn to imply that all ribosomes occur free in the cytoplasm instead of being generally attached to messenger RNA which can still be attached to the DNA.

Despite the usefulness of this new edition and the extensive revision that it has undergone, I think that more revision is still needed as the pace of discovery has been fast. Dubious or outdated items should be pruned, certain diagrams revised and the authors encouraged to bring out crucial features of selected experiments and to re-introduce a forward look.

Kenneth Burton

Kenneth Burton is Professor of Biochemistry at the University of Newcastle upon Tyne, UK.

Perspective on penicillin

Penicillin in Perspective. By David Wilson. Pp. xi + 298. (Faber and Faber: London, October, 1976.) £4.95.

It is now nearly half a century since Fleming's famous observation of the action of penicillin on a contaminated plate. This was followed, over a decade later, by the demonstration of its chemotherapeutic possibilities by a small group of workers at Oxford led by the late Lord Florey, which revolutionised the treatment of infection and triggered the explosive development of the multi-million pound antibiotics industry.

Because of its glamour and the ease with which the early work on penicillin can be grasped by the layman, several popular books on it or on antibiotics in general have appeared. Can yet another be justified? On reading the most aptly named *Penicillin in Perspective*, initial scepticism on this point is quickly replaced by respect for the thoroughness with which the author has researched his material and the

freshness of his approach; even those already familiar with the story will find more than a little that is new to them. The interweaving of the main theme with relevant background is skillfully done, and there are fascinating side-lights on personalities and circumstances. The part played by luck repeatedly recurs, and among a number of interesting speculations is whether, in these days of stringent official control of new drugs (perhaps partly in response to ill-informed public clamour) penicillin would ever have reached clinical medicine. There are a good number of quotations, some quite long, from original papers, from correspondence and from other books, which add interest and authority, and complement the author's compact but clear style.

It is good to find credit given to some of the little-known workers in the field, such as F. Ridley and (the late) Stuart Craddock, two medical students at St Mary's Hospital who, working under great difficulties, prepared a protein-free penicillin which could surely have been used for protective experiments in mice. Precisely why these crucial experiments were not attempted will never be known, but a fairly convincing explanation is offered in terms of the general scientific outlook at the time, the particular climate of opinion in Wright's laboratory, and Fleming's own character.

Few significant errors have been noticed: the barium salt of penicillin was not crystallised in 1941 (p183); penicillins I, II, III, and IV in the British nomenclature correspond to F, G, X and K respectively in that of the US (p16). A more serious inaccuracy is the claim (by the author) that the Oxford workers "found out how to manufacture penicillin on a commercial scale" (p141). It is true that they carried out culture of the fungus on what, for a research laboratory, was a very large scale, but the yield of penicillin was so painfully low (at most 2 units ml⁻¹) that the total production to the middle of 1941 and the completion of the trials on the first six parenterally treated patients at the Radcliffe Infirmary, was probably less than 5 megauunits. With modern high-yielding strains of the fungus in deep culture this amount could be obtained from less than half a pint of culture fluid!

This is indeed a worthwhile book and presents a sober, critical and well informed view of penicillin in perspective.

N. G. Heatley

N. G. Heatley was formerly Senior Research Officer, and is now Lecturer, at the Sir William Dunn School of Pathology, University of Oxford, UK.

announcements

Appointments

P. H. Parkin, of the Building Research Establishment, to be Visiting Professor and **J. S. Ellis**, formerly Professor of Orthopaedic and Accident Surgery, to be Emeritus Professor, both in the University of Southampton. **D. Phillips**, Senior Lecturer to be Reader in Chemistry, University of Southampton.

Professor Friedrich Deinhardt to the Chair of Hygiene and Medical Microbiology, and Director, Max v. Pettenkofer Institute, Ludwig-Maximilians University of Munich, from March 1, 1977.

The Clinical Research Centre at Northwick Park, Harrow has established three new divisions of Immunological Genetics, Perinatal Medicine and Rheumatology. The heads of these divisions are, respectively, **Winfred Watkins**, **F. E. Hytten** and **Barbara Ansell**.

Meetings

January 7, 1977, **The Drought of 1975-6**, London (Royal Meteorological Society, Grenville Place, Bracknell, Berkshire).

February 17, 1977, **Europe—the Directive and the Cosmetic Industry**, London (Society of Cosmetic Chemists, 56 Kingsway, London, WC2).

March 7-11, **Stability of Tropical Environments and Populations**, Panama (Dr R. T. de Araújo, P.O. Box 662, Panama 1).

March 31-April 1, **Semiconductor injection lasers**, Cardiff (Institute of Physics, 47 Belgrave Square, London, SW1).

April 18-22, **Molecular Beams**, Noordwijkerhout, Holland (Louise Roos, FOM-Institute for Atomic and Molecular Physics, Kruislaan 407, Amsterdam).

May 2-6, **Protides of the Biological Fluids**, Brugge, Belgium (Jerusalemstraat 34, B-8000 Brugge).

May 30-June 3, **American Geophysical Union**, Washington (AGU, 1909 K Street, N.W., Washington, DC 20006).

June 15-24, **Advanced Institute in Methods of Immunological Diagnosis**, Bloomfield Hills, Michigan (N. R. Rose, Wayne State University School of Medicine, 540 East Canfield, Detroit, Michigan, 48201).

Person to Person

Reading-Paris. Accommodation exchange for six months (approx.) from January/February 1977. We offer 3 large bedrooms, fitted kitchen, full central heating, garage and use of vehicle, if required, plus four other rooms. Quiet rural area, large garden. House is near to NIRD, Shinfield with easy access to Reading University. Anything in Paris considered for self and wife, preferably accessible to 16th Arrond. Write to c/o Wilkins, Spencers Wood Post Office, Reading, Berkshire, UK.

Frank Horne Memorial. A fund is being established to commemorate the former director of the National Institute of Agricultural Botany. Grants will be awarded from the fund for education and research in the production of seeds, plants and crops. Contributions and enquiries to The Secretary, NIAB, Huntingdon Road, Cambridge.

Adenia digitata Engl. Investigator would like to know a reliable source from which to obtain seeds, fruits and roots of this plant. Please write to Professor F. Stirpe, Istituto di Patologia generale, Via S. Giacomo 14, 40126 Bologna, Italy.

Furnished 4 bedroom house in Winchester, Massachusetts offered in exchange for 2-3 bedroom accommodation in Cambridge, England for four months, May-August, 1977. Winchester is within easy commuting distance of Harvard, M.I.T. and all major hospitals in Boston, and has an excellent school system. Dr Ajit Kumar, Shriners Burns Institute, 51 Blossom Street, Boston, Mass. 02114.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London Office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

June 18-19, **Gut Hormones**, Lausanne, Switzerland (S. R. Bloom, Department of Medicine, Hammersmith Hospital, Du Cane Road, London, W12).

June 27-30, **Genetics Society of Canada**, Winnipeg (Phyllis McAlpine, Department of Genetics, 685 Bannatyne Avenue, Winnipeg, Manitoba).

July 4-6, **Rare Earths and Actinides**, Durham (Institute of Physics, 47 Belgrave Square, London, SW1).

July 10-22, **Sensory Ecology (NATO Institute)**, Lennoxville, Quebec (M. A. Ali, Département de Biologie, Université de Montréal, C.P. 6128, Montréal, Canada).

August 2-5, 1977, **Cryogenic Engineering**, Boulder (Dee Belsher, National Bureau of Standards, Boulder, Colorado, 80302).

September 12-15, **Solid State Devices (ESSDERC 1977)**, Brighton (Institute of Physics, 47 Belgrave Square, London, SW1).

September 26-29, **Phase Transition in Bulk Polymers**, Varna, Bulgaria (M. Mihailov, Central Laboratory for Polymers, 1113 Sofia, Bulgaria).

Reports and publications

Great Britain—October

Waste Management Advisory Council. Paper No. 2: Report on Waste Paper Collection by Local Authorities. Pp. vi + 25. (London: HMSO, 1976.) 60p. net. [410]

Scholarships Guide for Commonwealth Postgraduate Students, 1977/79. Pp. 346. £3.75. Some Awards Open to Graduates of Foreign (non-Commonwealth) Universities and Tenable at UK Universities. Pp. 48. £1; \$3. (London: The Association of Commonwealth Universities, 1976.) [610]

Memoirs of the Royal Astronomical Society. Vol. 82, Part 2: Studies of A and F Stars in the Region of the North Galactic Pole—I. By G. Hill, A. Allison, W. A. Fisher, G. J. Odgers, E. L. Pfannenschmidt, P. F. Younger and R. W. Hilditch. Studies of A and F Stars in the Region of the North Galactic Pole—II. By R. W. Hilditch, G. Hill and J. V. G. Barnes. Pp. 69-94. (Oxford and London: Blackwell Scientific Publications, 1976. Published for The Royal Astronomical Society.) [710]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 282, No. 1311: A Study of Unsteady Forces at Low Reynolds Number—a Strong Interaction Theory for the Coaxial Settling of Three or More Spheres. By S. Leichtberg, S. Weinbaum, R. Pfeffer and N. J. Gluckman. Pp. 585-613. (London: The Royal Society, 1976.) UK £1.50; Overseas £1.60. [810]

The Royal Scottish Museum. The Scottish Society of the History of Medicine. The Early Years of the Edinburgh Medical School. Edited by R. G. W. Anderson and A. D. C. Simpson. Pp. 124. £3 net. Edinburgh and Medicine. Compiled by R. G. W. Anderson and A. D. C. Simpson. Pp. 72. £1.75 net. (Edinburgh: The Royal Scottish Museum, 1976.) [1110]

University of Oxford. Oration by the Vice-Chancellor and Annual Report, 1975/1976. Pp. 35-62. (Oxford: The University, 1976.) 20p. [1110]

The First Fifteen Years, 1961-1976: a Review of Advance in Scientific Research, and Replacement of Animals. Pp. 22. (Bramhall, Cheshire: The Humane Research Trust, The Lawson Tait Medical and Scientific Research Trust, 1976.) [1210]

Grown Over With Green-ness. By Deric Bolton. Pp. 48. (Walton-on-Thames: Outposts Publications, 1976. Available from Mr. F. J. Bolton, 17 Wester Coates Avenue, Edinburgh, EH12 5LS.) £1. [1510]

Other countries—October

- Proceedings of the Royal Irish Academy. Vol. 76, Section A, No. 12: Homogeneity and Extremely Convergent Spaces. By M. Fitzpatrick and S. D. McCartan. Pp. 111–116. 52p. Vol. 76, Section A, No. 13: Graphical Study of Rotational Brownian Motion. By G. W. Ford, J. R. Lewis and J. McConnell. Pp. 117–143. £1.65. (Dublin: Royal Irish Academy, 1976.) [1510]
- National Institute of Agricultural Botany. Fifty-sixth Report and Accounts, 1975. Pp. 96. (Cambridge: National Institute of Agricultural Botany, 1976.) [1810]
- Nigeria: Selected Studies. (Social Science Research for Population and Family Planning Policies and Programme). By John McWilliam and Chukwudum. (Research for Action, No. 1.) Pp. 50. Evaluation of Family Planning Programmes: An Example from Boswana. By Sheila Cook. Pp. 13. (Research for Action No. 2) (London: International Planned Parenthood Federation, 18/20 Lower Regent Street, SW1, 1976.) [1910]
- Guide to the Japanese and Korean Patents and Utility Models. By J. V. Drasil. Pp. 135. (London: The British Library, Science Reference Library, 1976.) £5. [2010]
- Asthma. Pp. 48. (London: Office of Health Economics, 162 Regent Street, W1, 1976.) 35p. [2110]
- Department of Energy. Fuel Efficiency Booklets. No. 5: Steam Costs and Fuel Savings. Pp. 13. No. 6: Flash Steam and Vapour Recovery. Pp. 13. (London: Department of Energy, Thames House South, Millbank, SW1, 1976.) gratis. [2110]
- National Radiological Protection Board. NRPB R46: Radiological Protection Standards in the United Kingdom. By Sir Edward Pochin, A. S. McLean and L. D. G. Richings. Pp. 5. (Harwell, Didcot, Oxon: National Radiological Protection Board, 1976.) [2110]
- Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 283, No. 1312: A Discussion on Natural Strain and Geological Structure. Organized by J. G. Ramsay, and D. S. Wood. Pp. 1–344 + plates 1–13. UK £20.50; Overseas £21.25. Vol. 283, No. 1313: The Absorption Spectrum of Europium. By G. Smith and F. S. Tomkins. Pp. 345–365 + 1 plate. UK £1.30; Overseas £1.35. B: Biological Sciences. Vol. 275, No. 942: Early Processing of Visual Information. By D. Marr. Pp. 483–524 + plates 1 and 2. UK £2.35; Overseas £2.45. (London: The Royal Society, 1976.) [2210]
- National Research Development Corporation. 27th Annual Report and Statement of Accounts, 1975–76. Pp. 48. (London: National Research Development Corporation, 66/74 Victoria Street, SW1, 1976.) [2210]
- Nuclear Prospects: a Comment on the Individual, the State and Nuclear Power. By Michael Flood and Robin Grove-White. Pp. vi + 64. (London: Friends of the Earth, Ltd., 1976. Issued jointly with National Council for Civil Liberties and the Council for the Protection of Rural England.) £1. [2510]
- Mineral Resources Consultative Committee. Mineral Dossier No. 16: Potash. Compiled by A. J. G. Notholt. Pp. v + 33. (London: HMSO, 1976.) 90p. [1510]
- Thames Water. Annual Report and Accounts for the year ended 31st March 1976. Pp. 24. (London: Thames Water Authority, New River Head, Rosebery Avenue, EC1, 1976.) [0000]
- The British Library: Science Reference Library. Periodicals on Animal Physiology held by the SRL. Pp. 50. (London: Science Reference Library, 25 Southampton Bldgs., Chancery Lane, WC2, 1976.) [2610]
- Procter and Gamble Ltd. Annual Report for the year ended 30th June 1976. Pp. 8. (Newcastle upon Tyne: Procter and Gamble, Ltd., 1976.) [2620]
- British Nutrition Foundation. Annual Report, 1975/1976. Pp. 26. (London: British Nutrition Foundation, 93 Albert Embankment, SE1, 1976.) [2710]
- Central Water Planning Unit. Annual Report 1975/1976. Pp. 66. (Reading, Berks: Central Water Planning Unit, 1976.) [2710]
- Tetrahedron Reports. No. 12: Réactivité des Hypobromites. Par P. Brun e B. Waegell. Pp. 11, £4.95; \$10. No. 13: 1-Acyl-2-Cyclopentenes and 5-Acylbicyclo (2.1.0) Pentanes: Photochemical and Thermal Isomerizations. By Kurt Schaffner. Pp. 13, £4.95; \$10. (Oxford and New York: Pergamon Press, 1976.) [2710]
- Natural Environment Research Council—Report of the Council for the period 1 April 1975–31 March 1976. Pp. v + 159. (London: HMSO, 1976.) £2.65 net. [2710]
- Headquarters Library, Department of the Environment. Register of Research 1976. Part 1: Building and Construction. Pp. iv + 227. Part 2: Environmental Planning. Pp. viii + 390. (London: Headquarters Library, Department of the Environment, 2 Marsham Street, SW1, 1976.) gratis. [2710]
- Headquarters Library, Department of the Environment. Register of Research 1976. Part 3: Roads and Transport. Pp. vi + 416. Part 4: Environmental Pollution. Pp. vii + 255. (London: Headquarters Library, Department of the Environment, 2 Marsham Street, SW1, 1976.) gratis. [2710]
- British Engine Technical Report 1976, Volume XII. Pp. 80. (Manchester: British Engine—Boiler and Electrical Insurance Co., Ltd., 1976.) [2810]
- The British Library: Lending Division. Directory of Library Codes. Interim edition. Pp. 42. (Boston Spa, Wetherby: The British Library, Lending Division, 1976.) [2810]
- Research supported by the Social Science Research Council, 1976. Pp. 493. (London: Social Science Research Council, 1 Temple Avenue, EC4, 1976.) £2. [2910]
- Whither R & D? (Proceedings of the 1976 Symposium held on 27th April at The Royal Society, London.) Pp. 71. (London: The Research and Development Society, 47 Belgrave Square, SW1, 1976.) £3 members; £6 non-members. [2910]
- Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem. Heft 169: Untersuchungen über die Morphologische und Biologische Differenzierung in der Fusarium-Sektion *Liseola*. Von Dr. H. Nirenberg. Pp. v + 117. DM 16.90. Heft 170: Xth International Symposium on Fruit Tree Virus Diseases 1976, Heidelberg. Pp. 117. DM 15.80. (Berlin-Dahlem: Biologischen für Land- und Forstwirtschaft, 1976.) [410]
- Proceedings of an International Meeting on Ecological Guidelines for the Use of Natural Resources in the Middle East and South West Asia, held at Persepolis, Iran, 24–30 May 1975. (IUCN Publications New Series, No. 34.) Pp. 231. (Morges, Switzerland: International Union for Conservation of Nature and Natural Resources, 1976.) \$6. [410]
- The Geological Survey of Canada: Past Achievements and Future Goals. By R. G. Blackadar. Pp. 44. (Ottawa: Energy Mines and Resources Canada, 1976.) [410]
- Environment Canada: Fisheries and Marine Service. Technical Report No. 636: The Culture of Brook Trout in Salt Water. By A. M. Sutcliffe, P. Harmon and H. Barchard. Pp. v + 21. Technical Report No. 649: Determination of Adenosine 5-Triphosphate in Estuarine Water and Sediments by Firefly Bioluminescence Assay. By D. J. Wildish. Pp. vii + 45. (St. Andrews, NB: Research and Development Directorate, Biological Station, 1976.) [410]
- Office de la Recherche Scientifique et Technique Outre-Mer-ORSTOM. Mémoires ORSTOM, No. 79: La Filariose de Bancroft en Afrique de l'Ouest. Par J. Brengues. Pp. 297. (Paris et Bondy: ORSTOM, 1975.) 75 francs. (410)
- United States Department of the Interior: Geological Survey. Water-Supply Paper 2130: Surface Water Supply of the United States, 1966–70. Part II: Pacific Slope Basins in California. Vol. 3: Southern Central Valley Basins. Pp. viii + 670. (Washington, DC: US Government Printing Office, 1976.) [510]
- Institut for Atomenergi, Kjeller. KR-153: Literature Survey—Health Effects of Radiation. By U. Tveten and K. Garder. Pp. 66. (Kjeller, Norway: Institutt for Atomenergi, 1976.) [510]
- United States Department of the Interior: Geological Survey. Professional Paper 955: Mineralogy and Geology of the Wagnerite Occurrence on Santa Fe Mountain, Front Range, Colorado. By Douglas M. Sheridan, Sherman P. Marsh, Mary E. Mrose and Richard B. Taylor. Pp. iv + 23. Professional Paper 968: The Paleontology of Rostroconch Mollusks and the Early History of the Phylum Mollusca. By John Pojeta, Jr. and Bruce Runnegar. Pp. iv + 88 + plates 1–54. (Washington, DC: US Government Printing Office, 1976.) [610]
- United States Department of the Interior: Geological Survey. Bulletin 1410: Structural Dislocations in Eastern Massachusetts. By Robert O. Castle, H. Roberta Dixon, Edward S. Grew, Andrew Griscom and Isidore Zietz. Pp. iv + 39. (Washington, DC: US Government Printing Office, 1976.) [710]
- European Organization for Nuclear Research—CERN. CERN 76-14: Short-Range Order and Local Conservation of Quantum Numbers in Multiparticle Production. By M. Le Bellac. Pp. 33. (Geneva: CERN, 1976.) [710]
- The Rockefeller Foundation. Working Papers. Food Production and the Energy Dilemma. By Ralph W. Cummings, Jr. Pp. 28. Conference on International Development Strategies for the Sahel. Pp. 500. Reaching the Developing World's Small Farmers. By Carroll F. Streeter. Pp. 48. Climate Change, Food Production and Interstate Conflict. (A Bellagio Conference, 1975.) Pp. 71. The Role of the Social Sciences in Rural Development. (An Inter-Agency Conference, 1975.) Pp. 56. The World Food Situation: A New Initiative. By Sterling Wortman. Pp. 35. Strategies for Agricultural Education in Developing Countries. Pp. 444. Strategies for Agricultural Education in Developing Countries. (Second Bellagio Conference, 1975.) Pp. 98. Food Crops in the Low-Income Countries: The State of Present and Expected Agricultural Research and Technology. By Ralph W. Cummings, Jr. Pp. 103. The Role of Animals in the World Food Situation. Pp. 101. (New York: The Rockefeller Foundation, 1974, 1975 and 1976.) [710]
- Information to Authors: Editorial Guidelines Reprinted from 140 Medical Journals, 1976–1977. Compiled by Harriet R. Meiss and Doris A. Jaeger. (New York: Gustave L. and Janet W. Levy Library, Mount Sinai School of Medicine of The City University of New York, 1976/1977.) [710]
- United States Department of the Interior: Geological Survey. Professional Paper 1002: The Guatemalan Earthquake of February 4, 1976, a Preliminary Report. Edited by A. F. Espinosa. Pp. ix + 90. (Washington, DC: US Government Printing Office, 1976.) [810]
- Publications of the Dominion Astrophysical Observatory, Victoria, BC. Vol. XIV, No. 18: The Spectroscopic Binary Characteristics of the Mercury-Manganese Stars. By G. C. I. Aikman. Pp. 379–410. (Victoria, BC: Dominion Astrophysical Observatory, 1976.) [1110]
- Report of the Sixth Session of the FAO Panel of Experts on Integrated Pest Control, held in Karachi, Pakistan, 20–23 October 1975. (Rome: FAO; London: HMSO, 1976.) £1.20. [1110]
- Nitrogen, Phosphorus and Sulphur—Global Cycles. Edited by B. H. Svensson and R. Söderlund. (SCOPE Report 7. Ecological Bulletins No. 22.) Pp. 192. (Stockholm: Swedish Natural Science Research Council, in collaboration with the Royal Swedish Academy of Sciences, 1976.) Sw. Cr. 40. [1210]
- Catastrophist Geology, Year 1, No. 1, June 1976. Pp. 1–48. Price for four quarterly issues Cr\$100 in Brazil, US\$10 outside Brazil. (Rio de Janeiro, Johan B. Kloosterman, Caixa Postal 41.003, 1976.) [1210]
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- Energy, Mines and Resources Canada. Geological Survey of Canada. Paper 75–45: Selected Bibliography on the Geology of Canadian Deposits and Occurrences of Uranium and Thorium. By D. M. Garneau. Pp. 41. \$2.40. Paper 76–4: Abstracts of Publications in Scientific Journals by Officers of the Geological Survey of Canada, April 1975–March 1976. Pp. 40. \$2.40. Paper 76–18: Geological Studies in St. Margaret's Bay, Nova Scotia. By D. J. W. Piper and M. J. Keen. Pp. 18. \$3. Paper 76–21: Sedimentology and Geochemistry of Marine Sediments Near Comox, British Columbia. By Kojin J. Clague. Pp. 21. \$2.40. (Ottawa: Geological Survey of Canada, 1976.) [1310]
- Argonne. 76. Pp. 32. (Argonne, Ill.: Argonne National Laboratory, 1976.) [1310]
- Memoirs of the India Meteorological Department. Vol. 32, Part 4: Rainfall of India Rainy Days of 1 Cent (0.3mm) or More. By K. N. Rao, C. E. J. Daniel and V. K. Bhargava. Pp. ix + 10. (Delhi: Controller of Publications, 1976.) [1310]
- Energy in Denmark 1990/2005: a Case Study. (Report No. 7—a Survey by The Work Group of The International Federation of Institutes for Advanced Study, c/o Sven Bjørnholm, The Niels Bohr Institute, The University of Copenhagen.) Pp. 57. (Copenhagen: The Niels Bohr Institute, Blegdamsvej, 17, 1976.) [1510]
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- Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Computing Research, 1974/1975. Pp. 64. Annual Report of the Division of Plant Industry, 1975. Pp. 140. Melbourne: CSIRO, 1976.) [1510]
- Indian Council of Medical Research. Annual Report of the Director-General, 1975. 153. (New Delhi: Indian Council of Medical Research, 1976.) [1510]
- India: Council of Scientific and Industrial Research. Technology Assessment. By H. Rothman. (Lecture Series, 1/75.) Pp. 45. (New Delhi: CSIR, 1976.) [1510]
- New Zealand Department of Scientific and Industrial Research: Geophysics Division. Seismological Report, 1973. Pp. 668. (Wellington: Seismological Observatory, Geophysics Division, 1976.) [1810]
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- Energy, Mines and Resources Canada. Geological Survey of Canada. Bulletin 255: An Atlas of Microfacies in Carboniferous Carbonates of the Canadian Cordillera. By Bernard L. Mamet. Pp. 131 (95 plates). \$10. Paper 74-49: Terrain Inventory and Quaternary Geology, Ashcroft, British Columbia. By J. M. Ryder. Pp. 17. \$3. Paper 75-33: Geology of Fort Graham E1/2 Map-Area, British Columbia. By H. Gabrielse. Pp. 28. \$3.60. (Ottawa: Geological Survey of Canada 1975 and 1976.) [2010]
- Australia: Commonwealth Scientific and Industrial Research Organization. Report of the Division of Building Research, 1974–1975. Pp. iii + 127. Annual Report of the Minerals Research Laboratories, 1975–76. Pp. 71. (East Melbourne: CSIRO, 1976.) [2110]
- France: Institut National de la Recherche Agronomique. Publication No. 6: Carte Pédologique de France a Moyenne Echelle. Condom, H.21. Notice Explicative par J. Seguy. (Service d'Etudes des Sols et de la Carte a Pédologique de France.) Pp. 152. (Versailles: Centre National de Recherches Agronomiques, 1975.) F.53.50. [2110]
- Australia: Commonwealth Scientific and Industrial Research Organization. Division of Soils. Technical Paper No. 27: A Water Balance Model and Supply Index for Wheat in South Australia. By E. L. Greacen and C. T. Hignett. Pp. 33. Technical Paper No. 28: Red Basaltic Soils in North Queensland. By R. F. Isbell, P. J. Stephenson, G. G. Murtha and G. P. Gillman. Pp. 49. (Melbourne: CSIRO, 1976.) [2110]